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A STUDY OF REDUCTION IN *OENOTHERA RUBRINERVIS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY III

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(WITH PLATES I–III)

The present contribution is a statement of some of the results obtained in the cytological study of *Oenothera Lamarckiana* and its mutants. Since these results have a more or less direct bearing on a wide range of fact and theory in various fields, their full discussion is reserved for a future time. The facts presented in this communication will be taken almost wholly from the study of *O. rubrinervis*, one of the most vigorous of the mutants. Other papers will be presented later, giving the further evidence upon which the conclusions of this paper rest, and attempting to indicate their bearing on the general problems of cytology and variation involved.

Material

The plants from which the material for these studies was obtained were grown from pedigreed seeds of DEVRIES, the purity of these cultures being further proven, in some cases, by carrying on the pedigree for another generation before collections were made. The results of these cultures, which are still being carried forward to later generations, will be published at another time in connection with an account of other studies on variation and hybridization in Oenothera. In this way it is hoped, if possible, to correlate the cytological data with the work in hybridization and variation. It seems to the writer that only by thus combining cytological with experimental studies is an explanation of the peculiar and remarkable phenomena of variation exhibited by the Oenotheras to be reached.

The cytological studies presented here will be confined chiefly to the phenomena of synapsis and reduction in the pollen mother cell. Various forms have been studied, a complete series of stages being obtained in some forms and a partial series or only a few stages being examined in others. The forms investigated include (1) *O. rubrinervis*, (2) *O. Lamarckiana*, (3) *O. gigas*, (4) *O. nanella*, (5) *O. biennis cruciata*, a variety of the European *O. biennis*, (6) both *O. lata* (see **12**) and *O. Lamarckiana* from the F_1 of *O. lata*×*O. Lamarckiana*, and (7) plants resembling *O. gigas*, from the F_1 of *O. lata*×*O. gigas*. Preliminary reports have already been made upon some of these studies, in various connections (**11, 12, 13, 14, 15**). Reference will be made to some of these results later.

The material from each individual was collected separately in nearly all cases, in order to observe possible individual differences in the same race, either in the number of chromosomes or in other cytological features. I am indebted to Mr. C. H. SHATTUCK for making a number of these collections. The material for the study of *O. rubrinervis* was obtained from a number of individuals grown in two different seasons and representing several strains derived from the same original pedigree. Sections were cut from six of these, and it may be stated here that in *O. rubrinervis* no individual differences were discovered, either in the number of chromosomes, which was 14 in all cases, or in any other features. In some of the other mutants, also, a number of individuals were examined. It was found necessary to reserve the account of *O. gigas*, which presents several features of special interest, for a separate paper. A preliminary report on this form has already been made (**14, 15**).

For various reasons, *O. rubrinervis* was chosen as the most favorable form for a thorough study of synapsis and reduction. The nuclei and chromosomes of Oenothera are small, and for this reason the selection of the most favorable type for study is a matter of some importance. In *O. rubrinervis* the pollen mother cells, although they vary much in size, are usually considerably larger than in *O. Lamarckiana*, the nuclei being also proportionately larger. The reason for this will be explained later. The chromosome number being low in most of the forms ($2x=14$, $x=7$), they can be counted without any difficulty, notwithstanding their small size. Another

notable advantage in comparing this with other studies in reduction is in the shape of the chromosomes, which are globular or somewhat oblong or cylindrical in most stages of mitosis, and are never greatly elongated or looped. For this reason it is a comparatively easy matter to obtain accurate counts of the chromosomes in the pollen mother cells of any of the forms. This shape is also found to be very advantageous in a study of the events of reduction following synapsis. The appearances are clear and easily interpreted, in striking contrast to the forms with long twisted chromosomes, such as have been made the basis of many of the studies on reduction in plants.

On the other hand, the somatic nuclei and chromosomes are very much smaller, and in metaphase the latter are elongated and looped, making it impossible to count them with the same degree of accuracy. Some of these appearances have already been described elsewhere (**12**, p. 19). Thus while it was found that the chromosomes could be counted almost equally well in pollen mother cells of all the forms studied, *O. rubrinervis* was found to be especially favorable for the investigation of reduction phenomena, especially the events of synapsis and the prophases of the heterotypic mitosis. The account given here will refer throughout to *O. rubrinervis*, with occasional comparisons with other forms. Later papers will take up these other forms in detail, in so far as this is necessary after the account presented here. Special attention will be given at that time, in particular, to the later stages, beginning with the telophase of the heterotypic mitosis, and also to the interesting conditions in some of the hybrids. The detailed account in *O. rubrinervis* will not be carried farther than the metaphase of the heterotypic mitosis, at which time the essential events have already taken place.

Methods

The usual methods of cytological technique were employed, various chrom-acetic and chrom-osmo-acetic solutions being tried until satisfactory fixation was obtained. The thickness of the sections varied from 4 to 10 μ. The latter thickness was found most favorable for counting chromosomes, because it is somewhat greater than the diameter of the nuclei, many of which in such sections were therefore uncut. It is possible to determine easily whether a nucleus has been cut by the knife by examining it in low and high focus. The

chromosomes in such uncut nuclei can then be counted with absolute accuracy, either in the prophase of the heterotypic mitosis before the disappearance of the nuclear membrane, or in the telophase after the walls of the daughter nuclei are formed. In nearly every individual examined, large numbers of such cases, all yielding the same result, were counted before the number was finally determined upon. The chromosomes could also be counted in certain positions on the spindle, particularly in anaphases, but in metaphase they were usually too closely grouped to allow of satisfactory counting.

In the second division, particularly in the forms having seven chromosomes as the gametophytic number, the chromosomes could be counted with certainty in almost any stage of mitosis. The thinner sections were used chiefly in the study of spirem and synapsis stages, although here also the comparatively short length of the thickened spirem frequently made it advantageous to study uncut nuclei in which the spirem could be followed throughout its length.

Of the various stains Heidenhain's iron-hematoxylin was found to be superior for chromosome counting and for clear differentiation of chromatic structures in nearly all stages of synapsis and reduction, safranin-gentian being used occasionally for comparison or for differentiating particular cytoplasmic structures. Orange G was also used with the iron-alum stain for bringing out clearly certain special features, particularly the protoplasmic connections between mother cells, which will be described later.

Description

EARLY STAGES

Some of the very early stages of the anthers, previous to the formation of mother cells, have been studied particularly with the purpose of tracing the origin of the bodies which were called heterochromosomes in my first paper. The provisional use of the name was based on the very close resemblance of these bodies to the chromosomes in appearance, and their frequent presence close by, or in some cases apparently attached to, the heterotypic spindle. They were not stated to pass undivided into one of the daughter nuclei, as misquoted by TISCHLER (**32**), but to remain outside in the cytoplasm where they gradually disappear. The study of their early history

shows that no line of distinction can be drawn between them and the large body readily recognized as the nucleolus. They are then smaller nucleoli, not differing essentially in origin from the single larger body which is almost constantly present in the mother cell during synapsis and prophase, but diverging from the latter somewhat in their later history.

In the earliest stages studied, the young meristematic cells of the anther primordia are very small (*figs. 1, 2*), and the tissues are wholly undifferentiated, except the epidermal layer. Usually several smaller nucleoli are present in each nucleus of the meristematic cells, in addition to the larger nucleolus. Compared with the cells of the anther wall when they are no longer meristematic, the smaller nucleoli of the former are about the size of the nucleoli of the latter, which are approximately equal in size. There is nothing in the latter corresponding to the larger nucleolus of the meristematic cells. Probably afterward one of these nucleoli enlarges as the cell increases in size, or it is possible that the nuclei of meristematic cells are always derived from previous ones which already possess a large nucleolus.

Chromatic staining bodies are also found closely appressed to the nuclear membrane in all the meristematic cells (*figs. 1, 2*). This tendency for chromatic material to accumulate on the nuclear walls gives these nuclei a characteristic appearance. These bodies often appear like a thickening of the membrane itself.

At the next stage studied many cell divisions have taken place, and the sporogenous, tapetal, and wall tissues have been differentiated. The sporogenous cells have increased enormously in size, and form a single row in longitudinal section down the center of the anther, the walls of these cells being especially thickened and distinct (*fig. 3*). The cells of the surrounding tapetal layer have also increased greatly in size and are distinctly marked off from the sporogenous row. In the sporogenous cells the nuclei (*fig. 4*), though much increased in size, have not increased in proportion to the cytoplasm. The large nucleolus, much larger than in the earlier stage, is now a conspicuous object in the nucleus. Smaller nucleolar bodies are almost invariably present, but masses are no longer found attached to the nuclear membrane. (The characteristic masses, however, may remain for some time attached to the nuclear walls of the tapetal cells).

Figs. 5–10 are from drawings of other nuclei at this stage of development. In the majority of cases one or two smaller nucleoli occur in addition to a single large one, but rarely (*fig. 6*) two large nucleoli of equal size may be found; and very frequently the number of small bodies, of equal or unequal size, may be greater, reaching as many as five or six. *Figs. 5, 7, 8, 9* show these in various stages of fusion with each other and with the large nucleolus.[1] They are thus not in any sense autonomous bodies. It appears that usually these fusions take place until only one large nucleolus and one or two smaller ones are present during synapsis and diakinesis. But occasionally the fusions do not take place, and several of these bodies may then be present in the later stages. The number of these nucleoli finally present depends, then, largely upon the amount of fusion which has previously taken place between them. In the later stages one large nucleolus is almost invariably present and usually a smaller one bearing a certain proportion to the larger in size, though the latter may vary in size and number as already stated. There is usually a clear area around the large nucleolus, as in the earlier stage, and threads of the reticulum may or may not cross this and appear to be attached to the nucleolus (*fig. 4*). The reticulum of the cytoplasm usually stains rather more deeply at this time than that of the nucleus. It may as well be stated at this time that in the resting nuclei of the pollen tetrad and in the nuclei of the nearly mature pollen grains of Oenothera one finds (*fig. 11*) the same condition of the nucleoli as in the mother cells, namely, usually one large and one small nucleolus bearing a rather definite size relation to each other, with sometimes additional small ones.

The sporogenous rows are differentiated from the tapetum by the greater growth of the cells, nuclei, and nucleoli of the former. At the same time they are distinctly marked off by the formation of a continuous thickened wall between tapetum and archesporium (*fig. 3*). It is obvious that as the cells and nuclei increase in size, the nucleolus grows also. Up to the time of synapsis the mother cells usually form a compact tissue, but about this time the cells begin to

[1] Miss NICHOLS (21) figures what are in all probability stages of fusion of large and small nucleoli in Sarracenia pollen mother cells, but interprets them as a budding-off of small bodies from the nucleolus. The budlike attachments to the nucleolus frequently observed by other authors are doubtless to be explained in like manner.

break apart at the corners where they meet, and before diakinesis is reached they are completely rounded off and independent, or they frequently remain connected with other mother cells only at the ends. In the meantime the cavity of the loculus grows rapidly, so that the mother cells, in normal development, usually lie loose in the cavity.

The events of synapsis and reduction usually go forward simultaneously throughout a flower, with comparatively little variation in the different parts of the same loculus or in the different anthers of a flower. In one flower, however, wide variation was found in different anthers, but comparative constancy in the loculus. One anther of this flower was in synapsis, another in diakinesis, another in metaphase of the heterotypic mitosis, and in still another some of the mother cells had completed the second mitosis. No abnormalities in the cytological condition of this flower were observed.

SYNAPSIS

After the stage described in *fig. 4*, the nucleus increases greatly in size, but without an appreciable increase in the size of the cell. The single row of sporogenous cells divides, so that there are usually two rows of pollen mother cells. Occasionally three or more mother cells are found in the cross-section of a loculus. In general there are fewer divisions than in the other forms, and this is at least one of the reasons why the mother cells are on the average larger than, for example, in *O. Lamarckiana*.

The resting nucleus of the pollen mother cell increases in size and begins to show signs of approaching synapsis. *Figs. 12, 13, 14* show stages in the beginning of this process. A number of these stages were found—although they are uncommon—in the same sections with regular synapsis stages. In some cases they occurred side by side with mother cells in which the synaptic knot had already been formed. A complete series of stages may be found in the same section, from the beginning of contraction to the formation of a close synaptic ball. The cytoplasm in these cells shows no contraction whatever, but is perfectly fixed. For this and other reasons there can be no doubt that this is a real contraction stage, leading to synapsis, and not a result of imperfect fixation, as one might judge on first examination.

That these nuclei are going into synapsis and not coming out is shown by several features: (1) the extremely delicate character of the threads, like those of the resting nucleus; (2) the fact that the periphery of the reticulum as it contracts frequently preserves perfectly the curved outline of the nuclear wall (*fig. 12*); (3) immediately after synapsis the thread is somewhat shorter and thicker than previously and appears to be continuous, while in the earlier contraction stages we still have the appearance of a reticulum (*fig. 13*).[2] As the contraction progresses, the threads are gradually rearranged from an anastomosing reticulum to a very long and continuous delicate thread. The exact manner of this rearrangement could not be observed, but one finds many transitions (*fig. 14*) from the anastomosing reticulum of the resting nucleus to the closely coiled and apparently continuous spirem of the synaptic knot (*fig. 15*). The contraction may take place from one side of the nucleus, leaving the reticulum attached for a time to the nuclear membrane at one point (*fig. 13*), or it may take place simultaneously from all sides (*fig. 12*). A few threads of the reticulum usually remain attached for a time to the nuclear membrane while the contraction is going on. These are drawn in finally as the synaptic ball becomes more compact.

The small number of these intermediate stages found indicates that they are passed through rather rapidly, the frequency of the occurrence of synapsis stages indicating, on the other hand, that this condition is of considerable duration.

No indication of a doubling or pairing of the threads during these intermediate contraction stages could be observed, though they were carefully searched for. Moreover, in the earliest stages of the synaptic ball the thread appears to be as thin and delicate as in the reticulum, which does not favor the view that a pairing has taken place. The evidence, then, so far as it goes, is decidedly not in favor of a pairing.

During these stages the nuclear membrane is often indistinct, making it difficult to define accurately the limits of the nucleus. The

[2] This explanation assumes, of course, that the synapsis stages themselves are normal and not due to artifact, as I presume all cytologists will now agree, although SCHAFFNER (**29**) apparently still entertains some doubt on the subject. The regularly coiled arrangement of the thread in the synaptic ball appears to me to be one of the best arguments against this stage being an artifact. Evidently a rearrangement of the threads is going on as contraction proceeds.

same condition is observed during synapsis, which is found in the same sections. In places the nuclear membrane has either disappeared or is too delicate to be observed. The cytoplasm, however, retains the original outline of the nucleus. MOTTIER (**20**) has apparently observed similar conditions of the nuclear membrane at this time. In some cases it is ruptured and a portion of it is actually carried inward with the nuclear reticulum at the beginning of the contraction (*fig. 12*). One is tempted to explain this as an artifact; but that this is not the explanation is shown by the considerations already mentioned. The explanation appears to be that as contraction proceeds a portion of the nuclear membrane may be torn away and carried inward attached to the threads. Frequently in these stages one finds the nuclear membrane present on one side of the nucleus but invisible elsewhere. This is the case in *fig. 12*, although the membrane was drawn as though complete. Observations of other nuclei bear out this interpretation, the nuclear membrane being clearly visible in some cases attached to portions of the reticulum which have contracted far away from the original position of the nuclear wall. In the late prophase, when the definitive chromosomes are formed, a distinct and perfect nuclear membrane is invariably present, so it would appear that in such cases as those just described a new membrane is afterward formed.

Mention must now be made of the chromatic staining material of the nucleus during these stages. The nucleolus is frequently, though not always, included within the synaptic knot. There is a tendency for other dark-staining bodies to accumulate near the periphery of the nucleus (*figs. 12, 14*); as contraction proceeds these are swept in by the reticulum. The exact relation they bear to the threads is not known. In some cases they appear, in the later stages of contraction at least, to form a part of the threads themselves, in other cases they appear to be merely inclusions in its coils. These bodies show no constancy in number, size, or shape. As the spirem takes on the appearance of the synaptic knot, they are still found in its meshes, and portions of the thread itself may also stain darkly, suggesting a solution of a part of their substance and its transfer into the thread. Even when the greater part of the spirem is completely decolorized certain portions of it retain the stain. This appears to be partly

due to the denser aggregation of the spirem in these regions, but in some cases it is evidently due to the presence of bodies which retain the stain and appear to be giving up the stainable part of their substance to the spirem. These bodies are evidently not the prochromosomes found by OVERTON (**22**) in certain dicotyledons, nor are they the gamosomes of STRASBURGER (**30, 31**).

Just the relation these bodies sustain to the spirem is not easy to determine. From *figs. 12* and *14* it is evident that they are at first small "nucleoli" caught in the contracting reticulum, but quite independent of it. Later they appear to give up a portion at least of their material to the spirem, finally disappearing as independent bodies. Usually, however, at least one of these bodies remains independent, and appears in synapsis and diakinesis as a small nucleolus bearing a definite relation to the size of the large nucleolus, being about the size of a chromosome. These bodies are usually free in the nuclear cavity (*fig. 15*). A certain depth of stain is required for demonstrating them during synapsis, for they usually decolorize more quickly than the large nucleolus. With a favorable stain they are found to be of strikingly uniform occurrence at this time. A plasma stain such as orange G may be used with advantage to demonstrate their presence. The uniformity in their occurrence is so great that for some time they were thought to be constant in size and number. With the demonstration of their inconstancy and their origin we have chosen to call them merely small nucleoli, as there appears to be no sufficient reason for another name. The (large) nucleolus disappears with great promptness immediately after the nuclear membrane breaks down, only persisting for a time in a few rare instances. In no case has fragmentation of the nucleolus, previous to its disappearance, been observed, although the presence of deeply staining globular bodies occasionally found near the periphery of the cytoplasm might be accounted for in this way. The mass of the latter, however, is sometimes greater than that of the nucleoli. The smaller nucleoli persist and are frequently found close by the heterotypic spindle. They may also be found on the homotypic spindle (*fig. 41*). Apparently they never reenter a nucleus, but remain in the cytoplasm until they finally disappear. These bodies have been found showing the same behavior in all the forms studied.

POST-SYNAPTIC STAGES

Synapsis lasts for a comparatively long time, as shown by the frequency of its occurrence in the material sectioned. During this time the spirem shortens and thickens and then begins to arrange itself more loosely in the nuclear cavity. This shortening and thickening is progressive (*figs. 16–18*) and apparently continues for some time. During these stages the thickness of the spirem may be nearly uniform throughout, or it may vary greatly, giving a moniliform appearance, or the spirem may appear irregularly constricted at varying intervals. In other cases, with a certain depth of stain it is seen to be composed of lighter and darker areas more or less regularly alternating. Portions of the thread may appear homogeneous or may show the lighter and darker areas, according to the depth of stain (*fig. 17*). In more deeply stained nuclei, such as *fig. 16*, the thread appears homogeneous throughout. These darker areas are the chromatin discs or chromomeres of various authors; and they give the thread a very characteristic appearance. During this well-defined stage the greatly thickened spirem is loosely distributed in the nuclear cavity. Deeply staining bodies still appear attached to or enmeshed in the coils of the thread.

At this time one finds undoubted indications of parallel threads. When represented by camera drawings in one plane the evidence for this is not so satisfactory as in the original preparation, but there is no doubt of their occurrence. As already stated, in the earlier stages previous to and during synapsis, parallel threads could not be observed, and it has not been determined whether they were really absent or whether the failure to observe them was due to their extreme delicacy. Hence it cannot now be stated whether they have arisen through an approximation of spirems at an earlier period, or through a split in the single continuous spirem. This matter will be discussed later.

Following this stage a second well-marked contraction takes place (*figs. 18, 20, 21*), apparently quite as typical and constant in its occurrence as the first contraction stage, which is ordinarily identified as synapsis. This contraction is of much shorter duration, however, and entirely different in appearance, owing to changes which the thread has undergone since synapsis, resulting in a great amount of shortening and thickening of the spirem. MOTTIER (**20**) has recognized this second contraction stage in Podophyllum, Lilium, and

Tradescantia, though he formerly thought it resulted from bad fixation; and it appears to have been observed also by FARMER and SHOVE (**10**). MOTTIER states that in these forms there is but little shortening of the spirem between synapsis and segmentation into chromosomes. In Oenothera, on the contrary, as is evident from a comparison of *figs. 15* or *16* with *22*, a very considerable amount of shortening as well as thickening of the spirem takes place during this interval. During the second contraction the paired threads apparently fuse, and further shortening of the (from now single) thread results in an enormous amount of thickening of the spirem, so that when it uncoils from this second contraction it has approximately the thickness of a chromosome and exhibits only a few loops. It can then frequently be traced through nearly its whole length. At this time there is a great amount of variation in the thickness of different parts of the spirem, as seen in *figs. 22* and *23*. *Fig. 19* is a portion of the spirem at this period, drawn with a higher magnification. It shows the chromatic bodies, which vary in size, imbedded in the linin substratum. As to how far two different substances are represented, I am at present unprepared to say.

DIAKINESIS

The single thick thread now segments transversely into 14 chromosomes, the sporophyte or *2x* number. At this time there is no indication whatever of a longitudinal split in the thread. Even when greatly washed out, the material of the chromosomes appears perfectly homogeneous, or if a granular structure is observable there is in its arrangement no indication of the previous split. At the time of this second contraction a pair of chromosomes is frequently observed separated from the spirem and apparently always lying with their long axes parallel and connected at one end (*figs. 20, 22*). This condition occurs very commonly, although in other cases the spirem is continuous throughout (*fig. 21*). In no case has more than one pair of chromosomes been observed to be thus precociously cut off in *O. rubrinervis*, though two such pairs have been observed in *O. lata* (see **11**, *fig. 19*). In no case has a single chromosome been observed to be cut off in this manner, and apparently they are invariably cut off in pairs, that is, bivalvent chromosomes are detached.

What significance this early separation of chromosome pairs may have is not known, but it appears that the later history of these pairs on the spindle can be traced. In the paper just cited (**11**), the writer wrongly identified them with the smaller nucleoli which persist by the heterotypic spindle. These chromosome pairs are frequently so closely approximated at the end opposite the end of actual connection as to give the appearance of a ring. It was thought that these rings by condensation (which actually takes place) were reduced to the size of these nucleolar bodies. The latter had the size and shape of chromosomes, and with a certain depth of stain invariably appeared hollow. These pairs are not condensed to rings, however, but to chromosome pairs of the ordinary Oenothera type.

The spirem at this time varies greatly in thickness in different parts, exhibiting constrictions and dilatations which indicate more or less clearly where segmentation into chromosomes will take place. This segmentation may happen while the spirem is still in the contracted condition (*fig. 25*), or after it has again uncoiled and distributed itself in the nuclear cavity (*figs. 24, 26, 28*), or before this uncoiling is completed. The segmentation appears to be in some cases nearly simultaneous (*fig. 24*); in other cases the segmentation is successive, as in *fig. 23*, where the spirem is clearly divided into three portions and the constrictions for the formation of the chromosomes are so far advanced that the number of chromosomes to be formed by each segment can already be foretold with practical certainty. The segmentation at this time is into 14 chromosomes, the sporophyte number. A large number of counts made at this time demonstrate the absolute constancy of this number in all the individuals of *O. rubrinervis* examined. It is possible, however, that individuals of this race may be found whose chromosome number differs from this number by one. This matter will be discussed later.

In every single case where the count could be determined with certainty it was shown to be 14. These counts were all made from sections 10 μ thick, and from nuclei which were uncut by the knife. The less numerous counts made in the multipolar stage of the heterotypic spindle gave invariably the same number. In this case all in a given cell were obtained by examining the adjacent sections. In all, hundreds of counts were made. In such nuclei as *figs. 26, 29, 30, 31*

there can be no possible doubt of the number of chromosomes present.

As already shown (*fig. 20*), one or in some cases more pairs of chromosomes may be cut off from the spirem before it undergoes segmentation, and frequently while it is still in the second contraction period. The exact method of origin of these pairs has not been observed, but they invariably, so far as observed, lie with their long axes parallel and connected at one end, from which it would appear that they were successive chromosomes on the spirem. In later stages, when the spirem has constricted into a chain of chromosomes arranged near the periphery of the nucleus, one or more pairs of chromosomes are found separated from the rest. Some of these have doubtless had the origin shown in *fig. 20*. Others appear to have originated later, as indicated in some of the figures, by successive chromosomes on the chain swinging around parallel to each other and thus pairing. Usually in diakinesis one or two such pairs are found, though occasionally there is no evidence of pairing. The highest number of pairs observed at this stage was five, with indications of pairing among the others (*fig. 29*); which is unusual. Later, in the multipolar spindle stage two distinct pairs are usually found in varying stages of conjugation (*figs. 35, 36*). A single case was observed (*fig. 37*) in which the fourteen chromosomes were all paired.

As the figures indicate, constriction of the spirem at regular intervals proceeds progressively until a chain of chromosomes is formed. When this has taken place, the chromosomes are at first several times longer than broad, and their margins have a very irregular, sinuous outline, like that of the spirem just previous to segmentation. They are not so long, however, that they can be twisted and looped in the confusing manner of many heterotypic chromosomes of plants. This is very gratifying in the study of these stages, since it permits a clearness of interpretation which would otherwise be unattainable. *Figs. 22* and *23* show the beginning of contraction, which has proceeded farther in *fig. 24*, leaving only the so-called linin connection between the chromosomes. The constrictions are all equivalent and the spirem thus segments into the sporophyte number of chromosomes and not into the reduced number of chromosome pairs. If successive chromosomes on the spirem are really the members of a pair, there is

nothing in the manner of segmentation of the spirem to indicate this. However, it is clear enough that one chromosome frequently swings around, as already mentioned, and pairs with its neighbor on the spirem. We do not really have, then, a transverse division of chromosome bivalents, but a separation of whole (somatic) chromosomes. Nothing has been found in the earlier stages which would correspond to the gamosomes and zygosomes of STRASBURGER, and even should a pairing of parallel threads during synapsis occur (a possibility which will be discussed later), the final pairing is between chromosome bodies which were lying end to end on a single spirem thread.

The linin connections during diakinesis appear to be merely the more finely drawn out portion of the spirem between the chromosomes. As condensation and contraction of the chromosomes progress, these linin connections become longer and more delicate (*figs. 31, 33*). The chromosomes become more dense and compact, being at first oblong-cylindrical (*figs. 24, 26*) and then more nearly globular or pear-shaped (*fig. 31*). Certain chromosomes sometimes undergo this contraction more quickly than others, as in *fig. 29*, and the different stages of this condensation may occasionally all be found in the same nucleus. In other cases the globular appearance is due to the position in which certain chromosomes happen to be lying (*fig. 34*).

HETEROTYPIC MITOSIS

During the prophase stages last outlined the cytoplasm usually possesses a more or less obscurely radiate appearance. A felt-work of fibrillae finally appears around the nuclear membrane. Later these fibrillae come to run tangentially to the latter, terminating in the cytoplasm, and by their aggregation in certain regions the multipolar spind'e is formed. From this stage the fibers are rearranged to form the bipolar spindle, passing through conditions in which the spindle appears quadripolar or tripolar in section. In the meantime the nuclear membrane has dissolved and the chromosomes are found at first in a cavity surrounded by fibers which preserve the outline of the nuclear wall. Later they come in and become attached to the chromosomes. Usually the large nucleolus has vanished before this time, but occasionally it may still be seen (*fig. 35*). In *fig. 37* the small nucleolus is shown, which can very frequently be seen at this

time. *Figs. 36* and *37* are merely sketches of the spindle fibers to indicate their general direction. *Fig. 35* is an unusual case. A cone of fibers appears to have been formed on one side only of the nucleus. The fibers are coming in and finding attachment to the chromosomes. The large nucleolus is still present, as well as two smaller ones.

The most critical stages of reduction have now been described and the remaining stages will be taken up with less detail at this time, but will be presented in full in a later paper. The chromosomes are at first irregularly arranged on the heterotypic spindle. As already seen, during spindle formation many of the chromosomes are frequently separate and unpaired. The attraction between the chromosomes which leads to pairing is evidently weak, so that it is doubtful if any pairing takes place at metaphase between chromosomes which had not previously paired. On the other hand, chromosomes which have once paired, no matter how early, appear to remain together until their separation in the metaphase of the heterotypic mitosis. Hence probably in many cases the chromosomes pass to the poles of the heterotypic spindle without having previously paired with each other, that is, they were merely lying loosely in the equatorial region of the spindle in metaphase, so that it was largely a matter of chance which pole any particular chromosome went to. This is believed to be a matter of prime importance in determining the final result of the reduction divisions in Oenothera, and the nature of the distribution of chromatin elements which takes place. Its possible significance will be pointed out in the discussion. *Fig. 38* shows the chromosomes just being drawn into the equatorial plate of the heterotypic spindle. In the examination of thousands of spindles in about this stage, one usually finds the chromosomes spread out in several planes along the long axis of the spindle. Of course some of these are early anaphase stages in which the chromosomes have begun their journey to the poles, but the condition is seldom found where the chromosomes are arranged regularly in pairs on the spindle. The daughter chromosomes seldom advance toward the pole in a single plane, as is the case in so many forms, but are more or less irregularly strung out along the spindle in their passage to the poles. This is in striking contrast with their behavior in the homotypic mitosis.

Usually in the early anaphase of the heterotypic mitosis a longi-

tudinal split appears in the daughter chromosomes. This split does not stop short of one end, giving a V-shaped body as in many plant chromosomes, but usually passes right through, forming two independent bodies, which, however, remain paired in the telophase and occupy a great variety of positions in regard to each other. The homotypic chromosomes thus assume many of the characteristic shapes which are usually observed in the heterotypic chromosomes of other forms, such as X, Y, V, H, etc. The failure of the heterotypic bivalents to form these shapes is due partly to the weaker attraction between the members of a pair, but largely to a difference in their shape, each member of a pair being usually more rounded in the heterotypic and more elongated and rodlike during the stages between the two mitoses.

The telophase of the heterotypic mitosis is one of the best stages for counting the chromosomes, as they are distributed at equal intervals around the periphery of the nucleus, no two ever being in contact and the halves of each (bivalent) chromosome rarely separating. The chromosomes now evidently repel each other, while the halves of each chromosome attract each other rather strongly. The halves of these bivalent chromosomes are usually short rods, but they may be dumb-bell or hour-glass shaped, or nearly globular, as previously mentioned (**12**). Sometimes, however, this split fails completely to occur in the anaphase, the daughter chromosomes remaining single and globular or somewhat elongated (*fig. 39*). These telophase stages and the prophases of the homotypic mitosis will be taken up in detail in a paper dealing with different forms. These results, therefore, will not be duplicated here, but a brief statement of the events of the second mitosis will be given.

HOMOTYPIC MITOSIS

In the telophase of the heterotypic mitosis the nuclei never pass into the resting condition and the chromosomes never lose their identity completely, though they spread out and anastomose with each other more or less. Nucleoli are formed, as previously described (**11**). These stages between the two mitoses last for some time, but the events of the second mitosis are passed through very quickly. The two homotypic spindles are formed simultaneously and their axes are

at various angles to each other. Spindle formation is the same as for the heterotypic mitosis, except that the spindles are smaller. In regard to the chromatin, suffice it at present to say that the chromosomes of the homotypic prophase show the same general types and are often identical in appearance with those of the heterotypic telophase. There can be no doubt that the bivalent bodies which appear on the homotypic spindle are the same bodies that were present in the telophase of the heterotypic. *Fig. 41* shows an early anaphase of the second mitosis, the members of each pair having just separated. One of the small nucleoli appears by one of the spindles.

IRREGULARITIES

In *fig. 39* spindle fibers are seen in the cytoplasm by the side of the spindle in anaphase. This may be connected with a condition which is illustrated in *fig. 40*. Six such cases were observed in which a regular spindle occurred at the side of the mother cell instead of between the daughter nuclei, after the partial or complete disappearance of the heterotypic spindle. Some of these cases were in the telophase of the heterotypic spindle (*fig. 40*); others were in the prophase of the homotypic. In these cases the spindles were regularly formed and rather sharp-pointed and occupied the same position at the side of the cell; of course they contained no chromosomes. The method of their origin is unknown, but it seems probable that they are connected with the condition observed in *fig. 39*. Mother cells which probably indicate an intermediate condition, in which irregularly arranged fibers were found at the side of the cell, were occasionally observed. They may merely indicate a persistence of the kinoplasm of the heterotypic spindle after its function has ceased, but their structure appeared remarkably definite in most of the cases observed.

A single case of extra nuclei in the pollen tetrad was observed in *O. rubrinervis*. These have been previously described in *O. lata* (**11**), where they are common occurrences in connection with pollen degeneration. The single case observed in *O. rubrinervis* is sketched in *fig. 42*. Two small nuclei are present in addition to the four larger ones composing the tetrad. The nuclei had passed too far into the resting condition to count the chromosomes in each nucleus.

POLLEN DEGENERATION

The general question of pollen degeneration in Oenothera is an interesting one. It reaches its extreme expression in *O. lata*, which is usually completely sterile in this regard, and in which I have already shown (**11**) that irregularities occur during the reduction divisions similar to those found in sterile hybrids. The question of sterility is evidently, as TISCHLER (**32**) suggests, a relative one.

In *O. rubrinervis* one is led from a gross examination to judge that the pollen production is copious and probably equal to that of *O. Lamarckiana* itself, but in reality many of the pollen mother cells fail to complete their divisions. From an examination of sections of anthers of *O. rubrinervis* it is found that in some loculi a large number or perhaps nearly all the mother cells may be degenerating in the synapsis stage. Frequently the cells are flattened and distorted, appearing pressed together for lack of space in the loculus. The chromatic contents of such cells often form a dense irregular mass, or their nuclei may be in normal synapsis or mitosis, notwithstanding the distorted shape of the cell; while still other cells of the same loculus may be entirely normal. Even earlier, in the archesporial stage, the tapetal cells in many sections were found to be breaking down, as in *O. lata* (**11**). No indications of degeneration have yet been observed in mother cells of *O. Lamarckiana*, and very few in the tapetum.

The percentage of mother cells which thus degenerate in *O. rubrinervis* was not determined. TISCHLER (**32**) suggests that the causes of sterility in mutants are the same as those in hybrids and in plants under cultivation. This general cause he designates as a disturbance or derangement of the constitution of the idioplasm, which he thinks has taken place in the production of mutants as well as in hybrids and under the conditions of cultivation.

PROTOPLASMIC CONNECTIONS

It is an interesting fact that large and rather conspicuous protoplasmic connections are found between the pollen mother cells in *O. rubrinervis*. They are usually quite easily seen and it is probable that they are always present. They consist of delicate strings or threads of cytoplasm connecting adjacent mother cells. In size they vary greatly, from the delicacy of a spindle fiber to a coarse thread or

strand connecting the cells (*figs. 45, 46*). When the cytoplasm has shrunken slightly away from the cell wall they are particularly clearly observable. These connections appear to be in all cases between mother cells, and in no case have they been observed between the mother cells and the tapetum. Generally one such strand is seen connecting two cells, but not infrequently there are two or three or occasionally even more. There is no constriction or change in the nature of the connective as it passes through the cell wall. These connections are even larger and more conspicuous in *O. gigas*, where the mother cells are also much larger. They have not been observed in *O. Lamarckiana* or the other forms, but they doubtless occur in all, being probably smaller and more inconspicuous in some.

Discussion

The method of reduction described in this paper at once raises a number of questions of prime importance from the cytological standpoint, as well as from that of the relation subsisting between hereditary and cytological phenomena. A discussion of all these features will not be attempted at this time, the intention of the writer being merely to indicate the general directions in which the facts point and the possible bearing which these data may be found to have on the problems connected with the phenomena of mutation in Oenothera. A fuller discussion of these subjects is reserved for a future time, after the presentation of further data. In the present communication reference will be made only to the most recent papers on reduction in plants, the purpose not being a review of the literature, or a discussion of present views, except in so far as they bear directly on the matter in hand.

The recent accounts of reduction in plants, given by BERGHS (**3, 4, 5, 6**), GRÉGOIRE (**16**), STRASBURGER (**31**), ALLEN (**1, 2**), MIYAKE (**18**), OVERTON (**22**), ROSENBERG (**25**), YAMANOUCHI (**33**), and others, have agreed in so far as the following general course of events is concerned: In synapsis a pairing of homologous maternal and paternal elements occurs either in the form of gamosomes (STRASBURGER and MIYAKE), prochromosomes (OVERTON), or parallel threads (ALLEN, ROSENBERG, GRÉGOIRE, BERGHS, CARDIFF **7**, and YAMANOUCHI). In every case two parallel threads result, which unite

more or less intimately about the time of synapsis or later. After the events of synapsis, a longitudinal split reappears in the thickened spirem threads, this split representing the line of approximation of the two original spirems. Transverse segmentation into pairs of chromosomes, which are believed to be homologous somatic chromosomes of maternal and paternal origin, then takes place. The halves of these bivalent chromosomes, which lie side by side, are then distributed in the heterotypic mitosis, which is thus a reduction division. In the anaphase of the heterotypic mitosis a longitudinal split appears in the daughter chromosomes, which is regarded as a premature split for the homotypic mitosis, the latter being thus an equation division. The persistency with which this general account has been given, notwithstanding differences in detail, particularly preceding and during synapsis, leads the writer to the belief that it is probably correct in its main outlines, at least in many of the forms described. This being judged to be the case, every effort was made to bring the account in Oenothera into harmony with this general course of events but without success, for Oenothera is found to deviate in some important particulars, as is already evident from the description.

Another general account of reduction in plants, which was adhered to by STRASBURGER as late as 1904 (**30**), and has been held notably by FARMER and MOORE (**8, 9**), FARMER and SHOVE (**10**), SCHAFFNER (**28**), MOTTIER (**19, 20**), and others, to mention only a few of the recent papers, is in general as follows: The split in the spirem which occurs at about the time of synapsis is a true split, such as may occur in the prophase of somatic mitoses, and is not preceded by a pairing of parallel threads, but the thread is single from the beginning. This split afterward closes up as the thread shortens and thickens after synapsis, and the single spirem so formed segments usually into the reduced number of chromosomes, which are thus arranged successively end to end. Each such bivalent chromosome thus consists of two halves arranged end to end, not side by side, and the heterotypic mitosis thus separates successive whole chromosomes on the spirem, being therefore, as in the other account, a reduction division. The split which appears in the anaphase of this mitosis is interpreted as a reappearance of the earlier longitudinal split of the spirem. The homotypic mitosis is therefore an equation or longitudinal division.

There are of course minor differences in these accounts, SCHAFFNER (**28**) stating, for example, that in *Lilium tigrinum* there is a splitting of granules in the spirem, but the linin thread remains single. Differences of opinion are also expressed regarding the arrangement of the loops of the spirem before segmentation, and their relation to the chromosomes formed.

These two general schemes agree that the heterotypic mitosis is a reduction division separating whole somatic chromosomes, while the second division is longitudinal. The essence of the distinction is that the first view regards the chromosome bivalents as formed by a side-by-side union of homologous chromosomes through the medium of parallel threads, while the second view holds to an end-to-end union. It will be seen that, omitting the points which are left undetermined, the account in Oenothera corresponds more nearly with the latter scheme than with the former, though differing in some respects from both. ROSENBERG (**25**), from a comparison of forms having long and short chromosomes, has attempted to harmonize the latter view with the former. He examined Listera, Tanacetum, Drosera, and Arum, and found that, for example in Drosera, which has short definitive chromosomes much like those of Oenothera, the spirem first segmented into long twisted chromosomes lying in pairs with their long axes parallel. Later, as they condensed into the short, rounded definitive chromosomes, they frequently swung around end to end, so that an observer seeing only the later stage would conclude that they had been arranged tandem on the spirem at the time of their origin. Similar conditions were sometimes observed in Listera. I think my *figs. 22–28* make it evident that this explanation will not apply to Oenothera. The chromosomes in Oenothera do not undergo any such great amount of condensation, but are already thick, heavy bodies when first formed from segmentation of the spirem (*fig. 24*). Their diameter at this time is about the same as that of the spirem just previous to segmentation, as is shown by comparing *figs. 22* and *23* with *figs. 24* and *26*. The fact that as many as eight or more chromosomes may be found forming a single connected chain (*fig. 26*) also renders this explanation impossible.

MIYAKE (**18**) finds that after the pairing of elements in synapsis (the exact method of this pairing need not be entered into here) in

Galtonia and Tradescantia, a longitudinal split appears in the thickened thread, and the double spirem thus formed breaks transversely into the reduced number of chromosome pairs. Later, in these forms, a secondary union between the chromosomes is claimed to take place, forming a single connected chain of chromosomes (as in Oenothera). Sometimes a pair of chromosomes lies free by itself at this time. Then by further shortening the chromosomes of Galtonia again fall apart into pairs, though in Tradescantia they frequently remain connected even after spindle formation. The apparent similarity of the chromosome chain thus described by MIYAKE in Galtonia to the condition in Oenothera, led the writer to make an endeavor to harmonize the two accounts. But instead of this, all the evidence obtained from a critical study of the stages concerned shows that in Oenothera a single very thick spirem breaks transversely into the sporophyte number of chromosomes. A critical examination of *figs. 22–28* will make it clear, I think, that we are following the progressive segmentation of a single spirem, and there is no room for stages between, in which a double spirem breaks into two parallel series of chromosomes. Moreover, it is hardly likely that secondary fusions between chromosomes would take place to such an extent as is shown in *figs. 23* and *24*. In nuclei such as *fig. 20*, in which a pair of chromosomes is cut off prematurely from the spirem while still in the second contraction, they are invariably connected at one end and rarely, if ever, at the other (though sometimes the close approximation of the latter ends may give the false appearance of a ring). This would not be the case if they came from separate paired threads merely lying side by side, so that this connection shows them to have been really successive on the spirem. From this evidence the writer cannot see how anything except a distortion of the facts can lead to the assumption in Oenothera of two parallel threads breaking into chromosomes. Hence the conclusion is that the double threads appearing in the stage represented by *fig. 17* have united to form a single thread, which then breaks transversely into the sporophyte number of chromosomes.

This corresponds fairly well with STRASBURGER'S 1904 (**30**) account of the post-synaptic stages in Galtonia, and suggests to the writer that perhaps after all the earlier account may be nearer the facts,

so far as the points here under discussion are concerned, than the paper of 1905 (**31**). The close similarity of the conditions in Gâltonia and Tradescantia during diakinesis to those in Oenothera suggests that they may be found finally to conform to Oenothera in these later stages. Whether or not this will be found to be the case, we must conclude that in Oenothera the longitudinal fission in the spirem (however it originated) closes up, and that after the second contraction, or during it, the thick thread segments into the sporophyte number of chromosomes. Since this diverges in important respects from nearly all the recent accounts of reduction in plants, the conclusion is that reduction probably takes place differently in different plants. Whether or not the results are different from the standpoint of a qualitative distribution will not be discussed now. The writer believes the above conclusions to be necessary, despite the fact that authors have reached different conclusions in regard to the same plant, particularly in such cases as Lilium and Podophyllum.

The next important point which requires discussion and which was left undecided in the statement of observations, is in regard to whether the double thread observed after synapsis arises from an approximation of parallel filaments or through a primary split in the thread. It may be well to examine the results which follow from either assumption. The writer hopes later to determine more definitely this difficult matter. On the first assumption of a lateral approximation in synapsis of two spirems representing respectively the maternal and paternal chromosomes, we should expect the double thread so formed to segment into the *reduced* number of chromosome pairs, in order to conform to the current account in forms in which there is a pairing of spirems, for example ALLEN (**1**), GRÉGOIRE (**16**), and YAMANOUCHI (**33**). Instead, however, the spirem segments into the unreduced number of bodies. We may still assume that each of these bodies consists of maternal and paternal longitudinal halves still closely held together and resulting from a previous approximation. According to this view the first mitosis would separate bodies which were arranged successively on the spirem, while the second mitosis would separate the maternal and paternal halves of these bodies. The reason for such a result would be that the maternal and paternal spirems remained closely fused after pairing, so that

their elements were separated in the second mitosis instead of the first. This view is scarcely admissible for several reasons. In the first place, on this hypothesis transverse segmentation of the spirem must have taken place not only between the (bivalent) chromosomes but also in the middle of each chromosome, in order to give a chain of fourteen bodies. Such a segmentation seems unlikely. Another possible explanation would be that the chromosomes have lost their identity during synapsis, and that the bodies we are dealing with now are new arrangements of the chromatic material, irrespective of the somatic chromosomes. Many considerations, however, strongly support the belief that these bodies really represent the somatic chromosomes. The facts so far educed in Oenothera, in the opinion of the writer, all favor the hypothesis of the separate existence and genetic continuity of the chromosomes from one generation to another. In this connection may be cited certain plants from the F_1 of *O. lata* $\times$ *O. gigas*, which as stated elsewhere (**14**) have 21 chromosomes as somatic number, 10 of which regularly go to one pole of the heterotypic spindle and 11 to the other. Occasionally, however, the segregated numbers of chromosomes are 12 and 9, one chromosome having gone to the wrong pole of the spindle. In this hybrid 7 of the chromosomes are maternal and 14 paternal. If in this case there were a pairing of maternal and paternal spirems, it is difficult to see how it could be accomplished and result in the distribution of chromosomes in the heterotypic mitosis already stated.

It will be instructive to compare the chromosome history in this cross with the often-quoted condition found by ROSENBERG (**23, 24**) in *Drosera longifolia* $\times$ *D. rotundifolia*. *D. rotundifolia* has 10 chromosomes and *D. longifolia* 20, as the gametophyte number. The hybrid naturally has 30 chromosomes in its sporophyte tissues, but in diakinesis 20 chromosome bodies appear, 10 of which are double, consisting of a larger and a smaller half, while the remaining 10 are the unpaired (smaller) *longifolia* chromosomes. The larger and smaller halves of the 10 bivalents separate and pass regularly to the poles of the heterotypic spindle, but the unpaired chromosomes are irregularly distributed or left out of the daughter nuclei. Later the pollen deteriorates. This result is strikingly different from that in the Oenothera hybrid, and, while perfectly in harmony with the

idea of the pairing of threads in synapsis in Drosera, makes it highly probable, and in fact necessary, that the method of reduction in the Oenothera hybrid be different. This is a strong argument not only against pairing of maternal and paternal spirems in Oenothera, but in favor of the probability that reduction takes place in diverse ways in the two genera. A considerable amount of time has already been devoted to the study of reduction in this Oenothera hybrid, and an account will be published later. So far as observed it shows no differences in method from the account given here for the pure races.

The hypothesis of the pairing of parental spirems in synapsis in Oenothera being thus rejected, the other alternative remains, namely, that the double spirem results from a split; and this appears to satisfy all the facts. The observations have already shown that the spirem segments into a single chain of chromosomes. The description of events in Oenothera from synapsis on thus agrees in outline with the 1904 account of STRASBURGER (**30**) in Galtonia, and in general also with that of FARMER and MOORE (**9**) in Lilium, Osmunda, Psilotum, and Aneura, FARMER and SHOVE (**10**) in Tradescantia, and MOTTIER (**19**, **20**) in Lilium, Podophyllum, and Tradescantia. The belief of the writer is that some of these forms will be found to correspond more nearly with the account which involves a pairing of threads, and some with the account involving only a split.

Another important matter which requires mention at this time is the nature of the chromosome distribution which takes place on the heterotypic spindle in Oenothera. As already observed, the chromosomes even during spindle formation are frequently unpaired. This appears to be due to the weakness of the mutual attraction which ordinarily leads to pairing. Granting that homologous maternal and paternal chromosomes unite when pairing takes place, what are the possibilities regarding the unpaired chromosomes? Pairing insures ordinarily that the members of the pair will proceed to opposite poles of the spindle, and hence that the homologous maternal and paternal elements will enter different nuclei. There is no such certainty in the distribution of the unpaired chromosomes, so that it might be expected that in certain cases both members of a pair would enter the same daughter nucleus. It is important to note that this result is entirely independent of the origin of these chromosome

pairs, whether from an end-to-end or side-by-side union of somatic chromosomes, or in any other manner, so that this question holds no necessary relation to the method of reduction. On the common cytological assumption that the chromosomes are qualitatively different (which has apparently been shown to be a fact in certain well-known cases in animals, that need not be cited), germ cells would occasionally arise lacking both members of a pair, and hence lacking the possibility of developing certain qualities. In this manner it is conceivable that a series of types might arise from the parent *O. Lamarckiana*, each lacking the possibility of developing a certain group of characters possessed by *O. Lamarckiana*.

On this view, which is suggested merely as a tentative hypothesis, we would have in the mutations of *O. Lamarckiana* an analytical process in which a series of types arises from the parent form, each lacking in a different group of qualities or capacities which the parent form possessed. This does not apply to *O. gigas*, however, which will be taken up at another time. The further bearings of this hypothesis on the mutation theory of DEVRIES will not be followed up in this discussion, but it may be pointed out here that such a hypothesis accounts for the absence of reversions of the mutants to *O. Lamarckiana*, and it may also account for some of the peculiarities of hybridization among the Oenothera mutants. I should therefore suggest that there may be a relation between the type of reduction in any organism and its variation and hybridization phenomena.

In Galtonia and probably also in Tradescantia there are apparently the same possibilities that both chromosomes of a pair may occasionally enter the same daughter nucleus. In other plant forms studied the attraction between chromosomes seems to be strong enough to keep the members of a pair together until their separation in the anaphase of the heterotypic mitosis. The segregation of the members of a pair into separate germ cells is thus insured. In cases where, as in Oenothera, the members of a pair do not always remain in contact, but are loosely arranged on the spindle, such a result as already suggested seems certain to occur in certain instances.

It has already been mentioned that occasionally one chromosome goes to the wrong pole of the heterotypic spindle. This is found to be the case particularly in the hybrids, for example, in the *O. Lamarcki-*

ana plants from the F_1 of *O. lata*×*O. Lamarckiana* (**13**), in which sometimes eight chromosomes pass to one pole and six to the other; but it may also occur rarely in the pure races. This matter was briefly discussed elsewhere (**14**). Assuming that the 14 chromosomes are in two similar sets of 7 each, and that homologous members of these sets conjugate except when there is a failure to pair, then when 8 chromosomes go to one pole and 6 to the other, both members of one of the pairs must have gone to the same pole. This probably takes place in cases where such members were unconjugated, for the purpose, or at any rate, the result of the pairing is in ordinary cases that one member of every pair shall be distributed to each pole. If, while two members of one pair thus go to one pole, the second member of another pair goes to the other pole, we should have an equal numerical distribution of chromosomes, but one daughter group would be lacking both members of one pair and the other would be lacking both members of another pair. It is highly probable that such a distribution occasionally takes place, though it would be less common than the case, already proved, where the members of a single pair are unilaterally distributed. It should be borne in mind that such cases are most likely to occur, not when the members of a pair are conjugated, but when they lie separately in diakinesis and on the spindle.

Miss LUTZ (**17**), from an examination of root tips, states that she has observed several individuals belonging to different strains having 15 chromosomes instead of 14. This is to be anticipated from the irregularities in chromosome distribution in reduction already mentioned. I have observed one such case in *O. lata*×*O. gigas* (**14**)—a certain plant having 20 chromosomes instead of 21. All the plants of *O. lata* (**12**) and *O. nanella* (**13**) thus far examined by me had 14 chromosomes, while Miss LUTZ (**17**) finds in root tips some *O. lata* plants with 14 and also some with 15 or she thinks possibly 16 chromosomes. She reports finding two *O. nanella* plants with 14 chromosomes and one with 15. Two *O. albida* seedlings are said to have 15 chromosomes and two *O. oblonga* plants 15, while a third has 14. Disregarding the possibility that these results might be due to the well-known variation in chromosome numbers in root tips, they are such as would be likely to arise in different individuals from the cytological irregularities I have already described. Whether there

are external differences between the plants having 14 chromosomes and those of the same race having 15, is as yet unknown. But it is quite conceivable that no such differences will be found, for if the sporophyte chromosomes consist of two complete sets (and for a variety of reasons this seems the only tenable view at the present time if we assume qualitative differences at all), the presence of an additional chromosome, which is already present in duplicate, would scarcely be expected visibly to affect the plant.

ROSENBERG (**26**) has found an analogous situation in Hieracium. For example, *H. excellens*×*H. Pilosella* gives hybrids with different numbers of chromosomes. This he ascribes to the fact that the eggs of *H. excellens* differ in their numbers of chromosomes, which he finds is due to irregularities in chromosome distribution during the reduction divisions. The writer has pointed out elsewhere (**12**) certain similarities between the hybridization phenomena in Hieracium and Oenothera, and this seems to be a further similarity between the two genera.

ROSENBERG (**27**) has since shown that *H. excellens* produces three kinds of embryo sacs: (1) Normal embryo sacs which require fertilization for their development. These are presumably the only ones which can be hybridized. The egg cells in these sacs vary in their number of chromosomes owing to the fact that some of the chromosomes, lacking in "affinity," remain univalent (that is, fail to pair) during the heterotypic mitosis and are irregularly distributed. It is evident that this lack of affinity between chromosomes is similar to that in Oenothera. (2) In rare cases apogamous embryo sacs are formed after a single division of the megaspore mother cell, and without reduction. (3) More frequently the condition occurs which ROSENBERG calls apospory, in which tetrad formation takes place and then an adjacent cell of the nucellus enlarges, displaces the tetrad, and forms an embryo sac without reduction.

Summary

In conclusion a brief summary of the facts and considerations here presented will be useful.

1. In Oenothera the heterotypic mitosis is a reduction division, separating whole chromosomes which lie successively on the spirem. The homotypic mitosis is an equation division, separating the longi-

tudinal halves of the daughter chromosomes of the heterotypic mitosis. Whether an approximation of threads or a split in a single thread occurs in synapsis was not determined with certainty from the observations, but various considerations lead to the belief that in Oenothera the doubling is due to a split which closes up later, rather than to an approximation of separate spirems.

2. The conclusion that the method of reduction probably differs in different genera is based on two considerations: (1) the fact that in most of the recent accounts of synapsis and reduction in plants a side-by-side pairing of chromosomes from maternal and paternal spirems is described, while in Oenothera the members of a pair are arranged end to end on a single spirem; and (2) on differences in chromosome distribution during reduction in certain hybrids of Drosera and of Oenothera (see p. 25). If reduction took place in the same manner in both genera, the chromosome distribution during reduction in these hybrids with reference to the parental chromosome numbers should be the same in both, but this is not the case.

3. Pairing between the definitive chromosomes during diakinesis and the prophase of the heterotypic mitosis does not always take place, owing to a weak attraction between the chromosomes. This allows irregularities of distribution in the heterotypic mitosis, so that both (unpaired) chromosomes belonging to one pair will *occasionally* enter the same daughter nucleus (see p. 26). Germ cells will thus arise, from which both members of a given pair of chromosomes are absent.

4. If we assume qualitative differences between the chromosomes or parts of them, various types would be expected to originate in this manner, each of them lacking the ability to develop certain qualities possessed by the parent form. On this view the mutations of *Oenothera Lamarckiana* are an instance of a process of analysis by which from the parent form arises a series of types, each lacking in certain characters or capacities possessed by the parent. This hypothesis would account for the absence of reversions among Oenothera mutants, and perhaps also for some of the peculiarities of hybridization in Oenothera. This matter will be considered at another time. This explanation does not apply to all the mutants, however; for example, *O. gigas*.

5. It is suggested that there is probably a direct relation between the events of reduction in a given genus and its variation, as well as its hybridization phenomena.

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EXPLANATION OF PLATES I–III

The figures were drawn with the aid of a Bausch & Lomb camera lucida. All except *figs. 1* and *19* were drawn under a Zeiss apochromatic objective 2^{mm} ap. 1.30, with a Zeiss compensating ocular 18. The figures are reduced one-fourth in reproduction, giving a magnification of nearly 3000 diameters. *Fig. 1* was drawn under a 2^{mm} objective and compensating ocular 6; *fig. 19* under B. & L. objective $\frac{1}{16}$ N. A. 1.32 and Zeiss ocular 18.

PLATE I

FIGS. 1, 2.—Young meristematic cells of anther primordium showing one large nucleolus and several smaller ones, and chromatic masses adherent to the nuclear membrane.

FIG. 3.—Longitudinal section of anther, showing size relations of nucleoli in sporogenous, tapetal, and wall cells.

FIG. 4.—One sporogenous cell from stage of *fig. 3*, previous to synapsis; cytoplasm somewhat vacuolate.

FIGS. 5, 7–9.—Nuclei at same stage, showing fusions of nucleoli.

FIG. 6.—Two nucleoli of equal size; an unusual condition.

FIG. 10.—Several small nucleoli, and no indication of fusion.

FIG. 11.—Nucleoli of young pollen grain nucleus.

FIG. 12.—Beginning of synaptic contraction; the reticulum has contracted from the nuclear membrane on all sides, leaving several loops attached to the membrane; on the side on which the reticulum retains the curved outline of the nuclear membrane the latter has been drawn inward attached to the threads; on the rest of the circumference, between the loops, the nuclear membrane remains *in situ;* the cytoplasm is perfectly fixed.

FIG. 13.—Another contraction stage, showing loops attached to the nuclear membrane, which is intact.

FIG. 14.—A slightly later stage of contraction, in which the rearrangement of threads is taking place.

FIG. 15.—Synapsis; dark-staining bodies are still held in the meshes of the spirem; a small nucleolus, usually about the size of a chromosome, is generally present in addition to the large nucleolus.

FIG. 16.—After synapsis; the thread thicker and shorter and loosely coiled.

FIG. 17.—Slightly later stage than *fig. 16*, and less deeply stained; thread shows the characteristic light and dark areas; indications of parallel threads in two places; edge of thread may be even or moniliform.—5 μ.

FIG. 18.—Later stage; thread much shortened and greatly thickened and entering upon second contraction phase; nucleus uncut.—10 μ.

FIG. 19.—Higher magnification of a portion of the thread in *figs. 20* and *21*.

PLATE II

FIG. 20.—Second contraction stage; a pair of chromosomes cut off from spirem; nucleus uncut.—10 μ.

FIG. 21.—Second contraction stage; nucleus uncut.

FIG. 22.—Uncoiling from second contraction stage; pair of chromosomes detached; nucleus uncut.

FIG. 23.—Spirem segmented in three places, each segment showing constrictions which will form the chromosomes; certain chromosomes already detached; nucleus uncut.

FIG. 24.—Constriction of spirem has proceeded farther, the chromosomes being elongated bodies with irregular margins like the spirem, and connected by rather

thick "linin" bands; pair of chromosomes detached earlier lies at side of nucleus; *n*, small nucleolus; nucleus cut.

FIG. 25.—Spirem more or less completely segmented into chromosomes while still in the second contraction stage; preparation considerably destained; 13 chromosomes in view.

FIG. 26.—Spirem segmented, showing chain of eight chromosomes and three pairs; nucleus uncut.

FIG. 27.—Chain of six chromosomes, and probably four pairs; linin connections between members of a pair not always visible; nucleus uncut.

FIG. 28.—Fourteen chromosomes; several small nucleoli; nucleus uncut.

FIG. 29.—Fourteen chromosomes, including five pairs more or less closely associated; linin connections not visible; one pair of chromosomes has already contracted into the globular shape.

FIG. 30.—Fourteen chromosomes, several in pairs; apparent inequalities in size due to positions in which some of the chromosomes are lying.

FIG. 31.—Slightly later stage; the fourteen chromosomes contracted into the globular or pear-shaped definitive form; linin connections longer and extremely delicate; nucleus uncut.

FIGS. 32–34.—Other groups in diakinesis, showing various peculiarities of chromosomes.

FIG. 35.—Peculiar case of spindle formation; three nucleoli present and fourteen chromosomes, including three or four pairs.

FIG. 36.—Multipolar stage of heterotypic spindle; two more or less closely united pairs of chromosomes present.

PLATE III

FIG. 37.—Same as *fig. 36;* an unusual case in which all the chromosomes are closely joined in pairs; seven such pairs present and a small nucleolus.

FIG. 38.—Heterotypic spindle in metaphase; spindle has usually more mantle fibers than in *O. Lamarckiana;* chromosomes usually loosely arranged in equatorial region of spindle.

FIG. 39.—Late anaphase; an uncommon case; daughter chromosomes have failed to divide, and fibrillae are scattered in cytoplasm at side of cell; chromatic staining material also present.

FIG. 40.—Telophase of heterotypic mitosis; exceptional case, in which a rather sharp pointed spindle is formed at side of cell; it probably originated from the fibrillae shown in *fig. 39*.

FIG. 41.—Early anaphase of homotypic mitosis; small nucleolus having the characteristic appearance, present on one of the spindles.

FIG. 42.—The single case of extra nuclei observed in *O. rubrinervis* pollen mother cells.

FIGS. 43, 44.—Nuclei from telophase of second mitosis, passing into resting condition.

FIGS. 45, 46.—Protoplasmic connections between mother cells.

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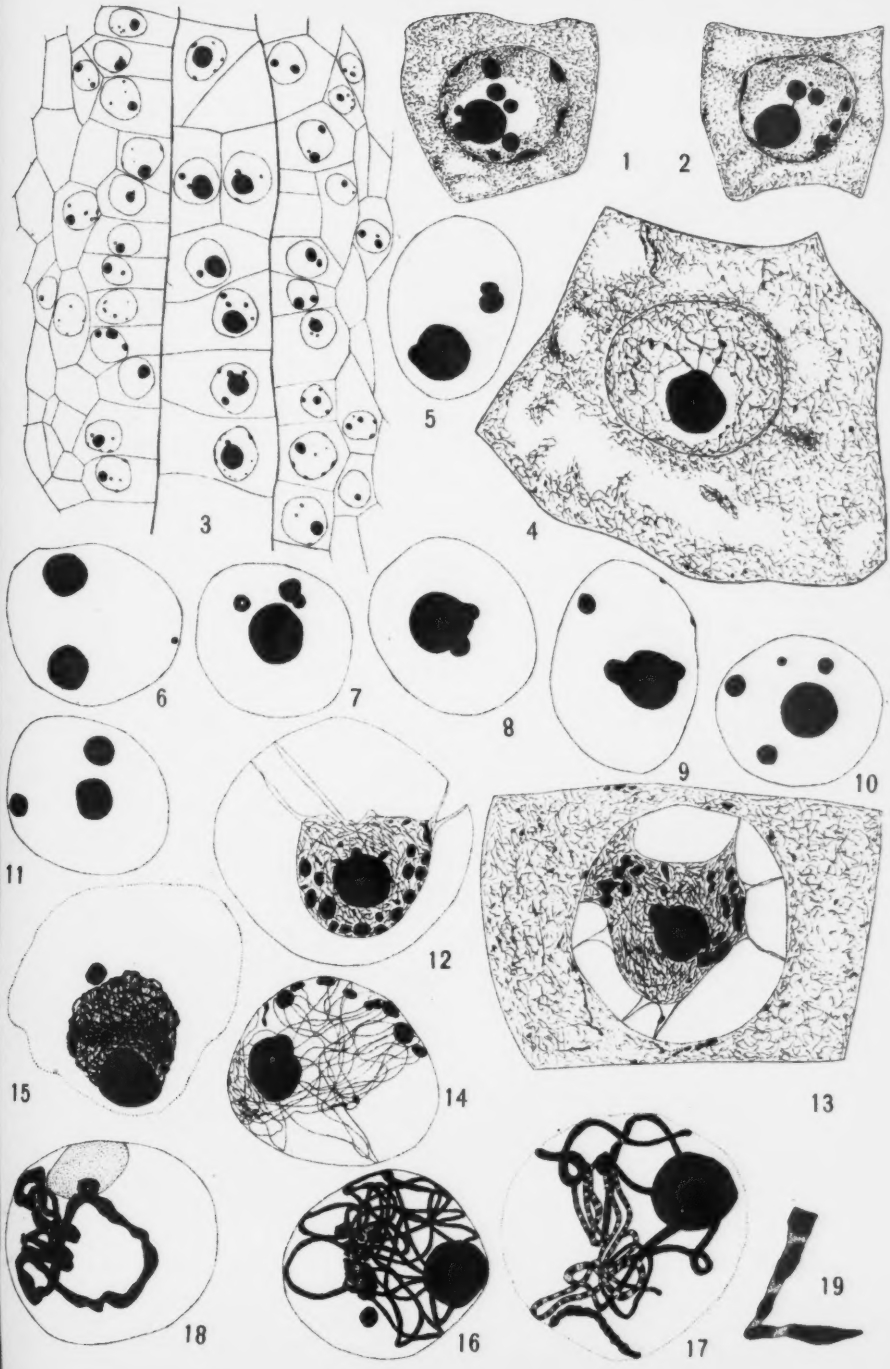

GATES on REDUCTION in OENOTHERA

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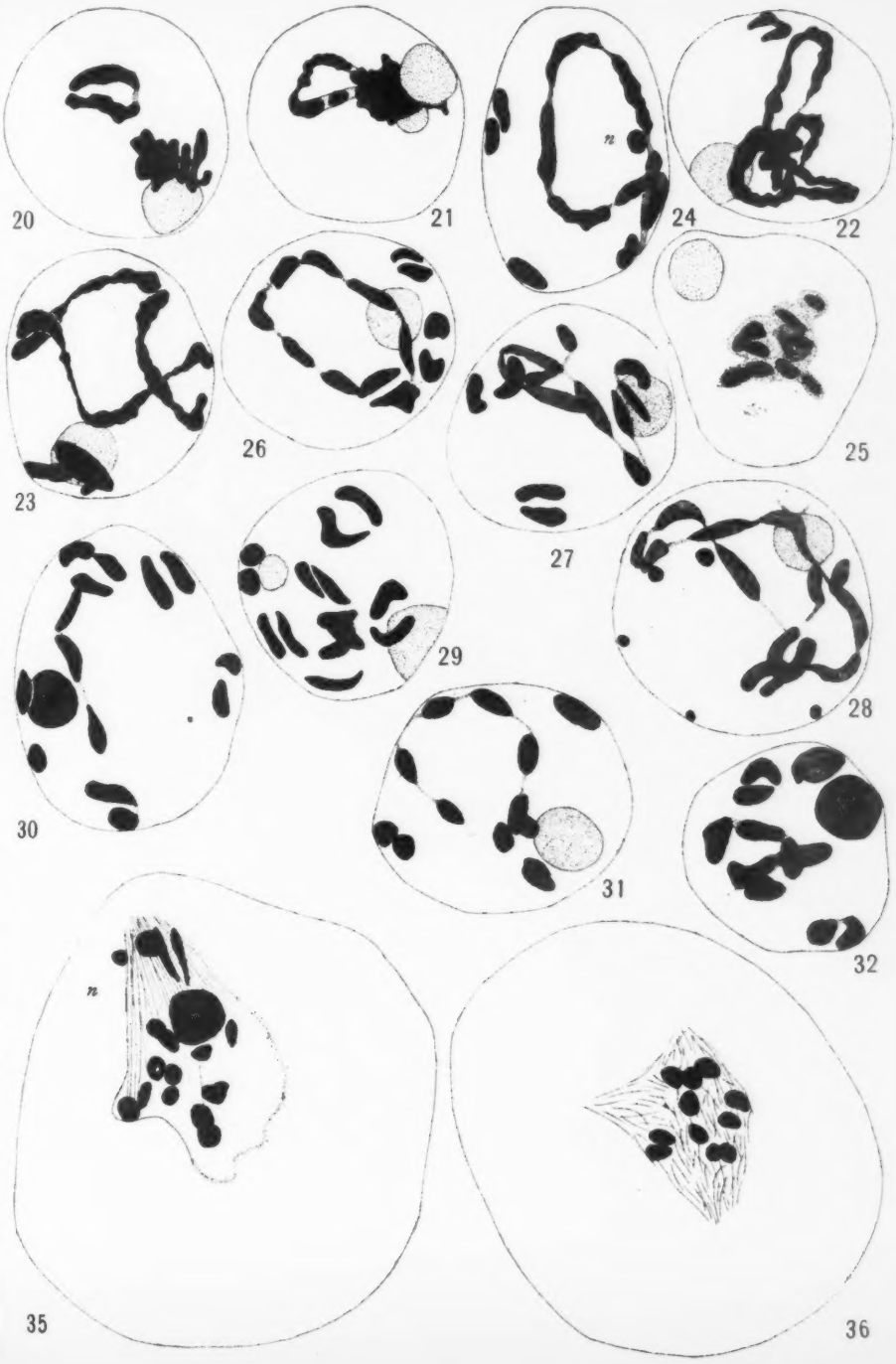

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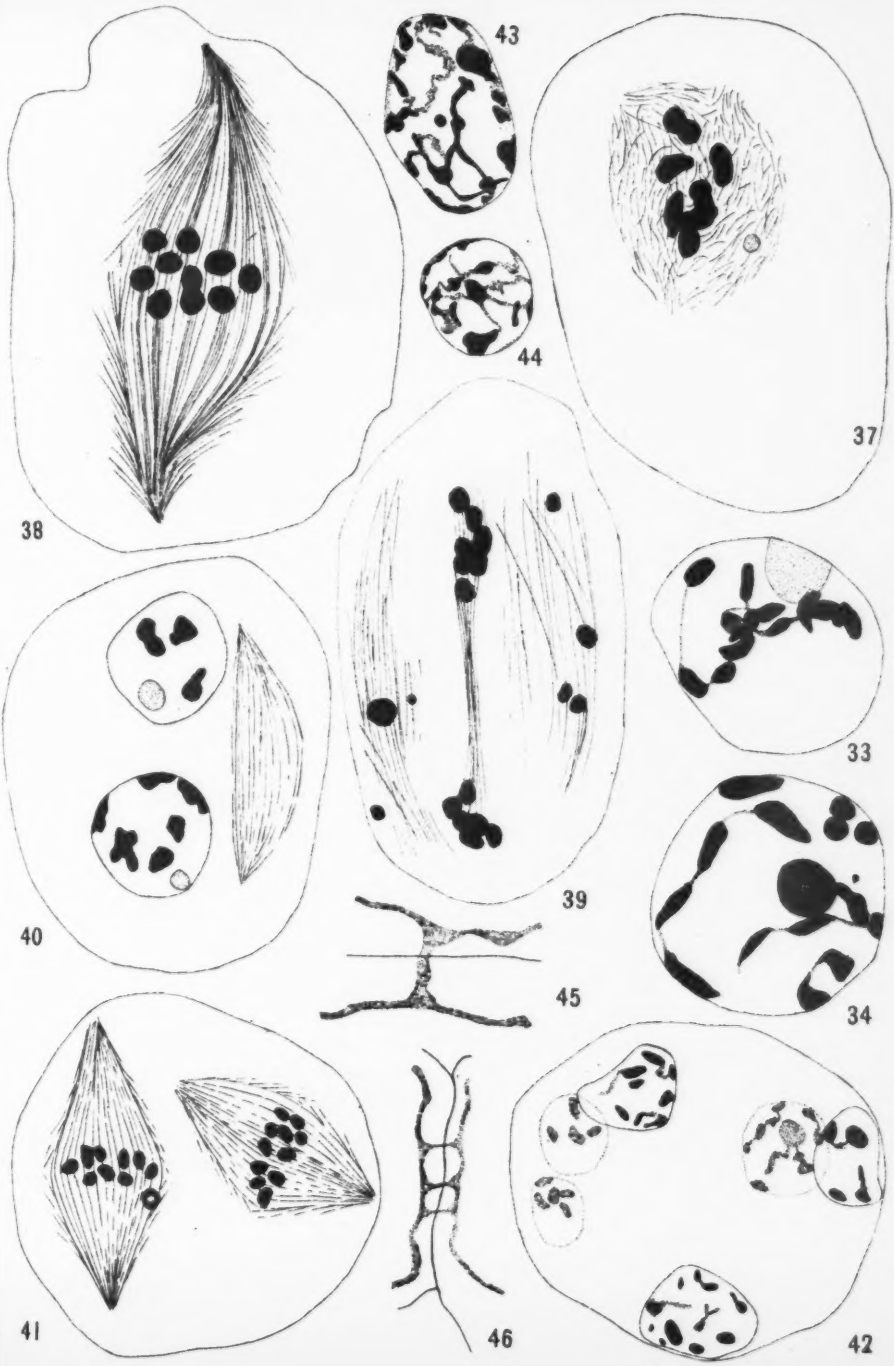


GATES on REDUCTION in OENOTHERA

STUDIES IN ARACEAE

JAMES ELLIS GOW

(WITH PLATES IV–VI)

I. NEPHTHYTIS GRAVENREUTHII

In the fall of 1906 the writer enjoyed the privilege of examining the extensive collection of tropical aroids in the greenhouses of the New York Botanical Gardens, and of collecting material for an investigation of the embryo sac and embryo. Among other species, *Nephthytis Gravenreuthii* was selected for this purpose. A considerable amount of material was obtained, illustrating stages from the archesporium to the mature embryo.

OVARY.—The genus is characterized by a single simple carpel which shows a tendency toward a slightly unsymmetrical development, the stylar canal never being in the axis of the carpel (*fig. 2*). The carpel is short and thick, the stylar canal very short, funnel-shaped, and lined with viscid conducting cells. On the interior of the carpel the conducting cells reach a considerable length and come in contact with the ovule on all sides, sometimes reaching quite to the micropyle.

OVULE.—The ovary contains a single, basal, anatropous, cauline ovule. Probably the single, basal, orthotropous ovule is the most primitive kind, and in Arisaema we find an orthotropous ovule, but there are typically four ovules; and while they are cauline in origin they occur as lateral outgrowths of a suppressed placenta, the ill-defined point of the placenta representing the axis of the flower. The single axial ovule, even though it be anatropous, is no doubt a simpler type than this; and when we compare it with such a form as Dieffenbachia, in which each ovule, arising from the partially suppressed placenta, is surrounded by a separate carpel, its primitive character becomes yet more apparent.

In general, the ovule of the species now under discussion is peculiar for its massive integuments and for the poor development of the nucellus. The latter consists at first of a single row of cells sur-

rounded by an epidermal layer (*fig. 1*). The hypodermal archesporial cell usually gives rise to two sporogenous cells; and one case of three sporogenous cells was discovered. In every case when observation was possible, the outer sporogenous cell developed the embryo sac. At the same time, the epidermal cells at the tip of the nucellus divide periclinally. No evidence was found to indicate that a primary parietal cell is ever cut off by the archesporium.

EMBRYO SAC.—As the ovule develops, the funiculus increases greatly in length, and this is accompanied by a corresponding lengthening of the outer integument (*fig. 2*). At the same time the integuments thicken considerably, and leave a narrow space between the nucellus and the inner integument. The embryo sac lengthens downward and encroaches somewhat on the chalaza (*fig. 3*). Preparations of sacs were obtained containing two, four, and eight nuclei. In the preparation represented in *fig. 3* (three sections of a single sac) there were eight active nuclei, but also traces of several degenerate nuclei, apparently indicating that more than eight nuclei had been formed. In *fig. 3a* there appears to the left a small nucleus with feebly defined boundary, but abundant chromatin contents; two similar but smaller nuclear fragments appear in *fig. 3b*. All this perhaps indicates the presence of ten or twelve nuclei in the embryo sac, some of which break down.

Three of the eight active nuclei form the egg-apparatus (*fig. 4*). Fusion of the polar nuclei was not demonstrated, nor were any of the phases of fertilization well shown except in one case (*fig. 12*).

The number of antipodals is variable; for example, there are three in *fig. 5*, two in *fig. 6*, and four in *fig. 11*.

ENDOSPERM.—The endosperm begins with free nuclear division. Whether it originates from a single endosperm nucleus, or by direct divisions of six of the eight nuclei previously figured could not be satisfactorily determined. In *fig. 5* walls are beginning to appear between the free nuclei and it seems that the wall-formation begins at the middle of the sac and proceeds toward the ends.

The walls of the endosperm cells soon become heavy (*figs. 6, 7, 8*). When the embryo reaches the stage shown in *fig. 8*, the thickening of the endosperm walls is complete, and the nuclei begin to show a tendency to disintegrate.

EMBRYO.—The first divisions of the fertilized egg were not found. The earliest stage observed is shown in *figs. 6* and *8*, showing a spherical proembryo without a suspensor. The proembryo is usually at the upper extremity of the sac, but its position is extremely variable. *Fig. 9* represents a somewhat older embryo, a slight notch beginning to show on one side, which at later stages becomes more pronounced (*fig. 10*). At the same time the endosperm is gradually destroyed, and the embryo finally assumes the form shown in *fig. 11*, the endosperm having disappeared completely.

POLYEMBRYONY.—The sections shown in *figs. 6*, *7*, and *8* were cut from the same sac. *Fig. 6* shows the embryo developed from the fertilized egg, and also a synergid; while in *fig. 8* another embryo is shown. These two sections cannot be different sections of the same embryo, for there are several intervening sections which show endosperm only, one of which is represented in *fig. 7*. It should be noted also that the second embryo (*fig. 8*) is not lower down in the sac than the one shown in *fig. 6;* in fact, the two lie side by side. All of the sac lying above the embryo in *fig. 8* represents a lateral upward extension of the sac not shown in *fig. 6*. This would suggest that the second embryo may have been derived from a synergid.

2. DIEFFENBACHIA DARAQUINIANA

In the material collected in the greenhouses of the New York Botanical Gardens, some was obtained from specimens labeled *Dieffenbachia daraquiniana*. This specific name is recognized neither by SCHOTT nor ENDER, and the species is probably identical with the one listed by the latter as *D. baraquini*.[1] The genus is notable in that the spadix is adherent along one side to the broad spathe, in which it is more or less tightly inclosed, the flowers occurring on the opposite side of the spadix. In the species under discussion the flowers were found scattered irregularly over the free surface of the spadix, crowded in places, but usually standing far apart and showing between them bits of the surface of the spadix. The male flowers occupy the upper part of the spadix, the female flowers the lower, the latter consisting only of an ovary. No traces of floral envelopes

[1] ENDER, Index Aroidearum 43. Berlin. 1864.

are present, but many staminodia are inserted near the base of the ovaries.

OVARY.—In the youngest material examined, the beginning of the flower appears as a slight protuberance on the surface of the spadix. A transverse crease soon appears, dividing this into two ill-defined lobes (*fig. 13*) which are destined to develop into the two carpels, and between the carpels appears the placenta, representing the axis of the flower (*fig. 14*). The carpels rapidly increase in size and overtop the placenta (*figs. 15, 16*). At the same time the ovules appear as lateral outgrowths of the placenta, filling the two cavities formed by the growth of the carpels (*fig. 15*). The continued growth of the carpels causes them to approach each other until there is left between them only a narrow canal, which divides over the apex of the placenta in such a way that the two branches lead down into the cavities of the carpels (*fig. 17*). On the upper and inner surface of each carpel, and leading down to the stylar canal, occurs a circular patch of somewhat viscid stigmatic cells (*fig. 17*).

OVULE.—The ovule first appears as a dome-shaped mass of undifferentiated cells projecting laterally into the carpellary cavity (*fig. 15*). The inner integument soon appears as an ill-defined ring about the upper portion of the ovule, and by the time the carpel has partially covered the placenta, the integument has grown out even with the apex of the nucellus, and the outer integument has begun to appear (*fig. 16*). At this stage the ovule is orthotropous. The lower surface now ceases to develop, while the upper continues to grow, thus finally making the ovule anatropous. At the same time, the integuments lengthen greatly, the outer one much exceeding the inner one (*fig. 17*).

ARCHESPORIUM.—Before the ovule becomes anatropous, the archesporial cell may be recognized by its greater size and by its nucleus (*fig. 19*). Before the first division of the archesporium the overlying epidermal cells usually divide once (*fig. 20*). The archesporial cell, which in this case is the mother cell, gives rise to four megaspores (*fig. 21*), the outermost one of which produces the embryo sac.

EMBRYO SAC.—The functioning megaspore increases greatly in size, destroying as usual the other megaspores and some of the

adjacent tissue. When the outer integument has closed over the nucellus, the first nuclear division in the embryo sac usually occurs (*fig. 22*). This is followed by the usual divisions (*figs. 23, 24, 25*), until eight nuclei are formed, which assume the usual positions. In this case the polar fusion was observed (*figs. 24, 25*). Before the embryo sac is complete, the lateral tissue of the nucellus has usually disappeared, only the tissue at the tip remaining.

FERTILIZATION.—Although the material was at the proper stage to show fertilization, good preparations were very difficult to obtain. The best are shown in *figs. 26, 27, 28*, and double fertilization is evident.

ENDOSPERM.—Before the first division of the fertilized egg takes place, the embryo sac has increased greatly in size and has become almost completely filled with endosperm. The endosperm begins with free nuclear division, which continues until numerous nuclei are distributed through the sac. Later wall-formation occurs (*fig. 28*) and the sac is filled with tissue.

EMBRYO.—At the close of the free nuclear stage of the endosperm the fertilized egg divides transversely (*figs. 29, 30*); then longitudinal and transverse divisions follow in no definite sequence until the spherical proembryo is produced (*fig. 28*).

CHROMOSOMES.—The preparations of Dieffenbachia were unusually favorable for a definite count of chromosomes, and the alternating numbers were found to be eight and sixteen.

3. AGLAONEMA VERSICOLOR

Flowering material of this East Indian species was also obtained from the New York Botanical Gardens. The earlier stages of the microsporangium were not found, but quite a complete series was obtained showing the development of the megasporangium.

MICROSPORANGIUM.—In the youngest material examined the tapetum and middle wall layers had disappeared, leaving only the distinct endothecium overlaid by the epidermis. At this stage the mother cells have rounded off and are forming the tetrads. In older material the division into tube and generative nuclei was observed and eight chromosomes were counted (the reduced number).

MEGASPORANGIUM.—Aglaonema has one carpel containing a single anatropous ovule, which is cauline, although its lateral position at

maturity might suggest that it is carpellary in origin (*fig. 33*). The stigmatic surface is prominent, and the cavity of the stylar canal is partially filled by a mass of similar cells, and long conducting cells extend downward on either side of the ovule, reaching the vicinity of the micropyle. At an early period the inner integument closes over the tip of the nucellus, but the outer integument does not close over the inner (*fig. 33*).

The earliest satisfactory preparations of the embryo sac show the first division (*fig. 36*) and the second (*fig. 37*). The final stages show great irregularity. *Fig. 38* shows a group of three cells (to the right) which suggests the egg apparatus; the two cells at the extreme left are certainly antipodals; while the two in the middle are evidently fusing polar nuclei. The solitary cell to the left suggests the third antipodal, but a male cell appears to be fusing with it. The ninth cell may be the other male nucleus. The embryo sac represented in *fig. 39* contains only five cells, two of which are clearly fusing polar nuclei.

The number of antipodals varies from two to eleven, and it is quite possible that they may sometimes be even more numerous (*figs. 33, 34, 35, 40*).

ENDOSPERM.—In Aglaonema the endosperm does not begin with free nuclear division, as in Dieffenbachia, Nephthytis, and Arisaema, but wall-formation begins at once. The growing endosperm first lines the side of the embryo sac which is toward the funiculus, but later fills the cavity and completely surrounds the embryo (*figs. 33, 40, 41, 42*).

EMBRYO.—The embryo much resembles that of *Lysichiton kamschatcense* as figured by CAMPBELL.[2] *Fig. 41* represents the position in which the proembryo was most frequently found, but in many cases it appears at the micropylar extremity of the sac. It appears quite as frequently adhering to the surface opposite the growing endosperm, and in at least one case was found resting on the antipodals. There was no evidence of displacement by sectioning, so that this variable position is probably due to the fact that the egg may lie in any part of the embryo sac.

BLAIRSTOWN, N. J.

[2] CAMPBELL, D. H., Notes on the structure of the embryo sac in Sparganium and Lysichiton. BOT. GAZETTE **27**:153–166. 1899.

EXPLANATION OF PLATES IV–VI

PLATE IV

Nephthytis Gravenreuthii

FIG. 1.—Section of tip of ovule, showing massive integuments, small nucellus, and two sporogenous cells.

FIG. 2.—Ovary at somewhat later stage.

FIG. 3.—Three adjoining sections of the same embryo sac, showing eight vigorous nuclei and traces of two or three others.

FIG. 4.—Egg-apparatus.

FIG. 5.—Antipodals, and endosperm nuclei with incipient wall-formation.

FIG. 6.—Endosperm at a later stage, proembryo (*e*), and a persistent synergid (*s*).

FIG. 7.—Endosperm.

FIG. 8.—Second proembryo in same sac shown in *fig. 6*.

FIG. 9.—Proembryo.

FIG. 10.—Embryo showing notch.

FIG. 11.—Mature embryo.

FIG. 12.—Fertilization, showing pollen tube (*p*), egg (*o*), male cell (*m*), and synergid (*s*).

PLATE V

Dieffenbachia daraquiniana

FIG. 13.—First appearance of carpels.

FIG. 14.—Later stage.

FIG. 15.—First appearance of ovule.

FIG. 16.—Later stage, showing nucellus (*n*) and integuments (*i*, *o*).

FIG. 17.—Section of ovary, showing stigmatic surface (*st*), stylar canal (*s*), and the anatropous ovules.

FIG. 18.—Section of ovule showing an embryo sac containing endosperm nuclei (*e*); the other structures are evident.

FIG. 19.—The archesporial cell.

FIG. 20.—Division of epidermal cells.

FIG. 21.—The linear tetrad, the outermost megaspore (*m*) functional.

FIGS. 22–25.—Stages in the development of the sac.

FIG. 26.—Fertilization of egg.

FIG. 27.—Fusion of male cell with endosperm nucleus.

FIG. 28.—Free endosperm nuclei.

FIG. 29.—Endosperm at later stage, and proembryo.

FIGS. 30–32.—Early divisions in proembryo-formation.

PLATE VI

Aglaonema versicolor

FIG. 33.—Ovary with the solitary ovule; embryo sac containing embryo (*e*), endosperm (*n*), and antipodals (*a*).

FIG. 34.—Group of eleven antipodals.

FIG. 35.—Group of three antipodals.

FIG. 36.—First nuclear division in embryo sac.

FIG. 37.—Four-nucleate sac.

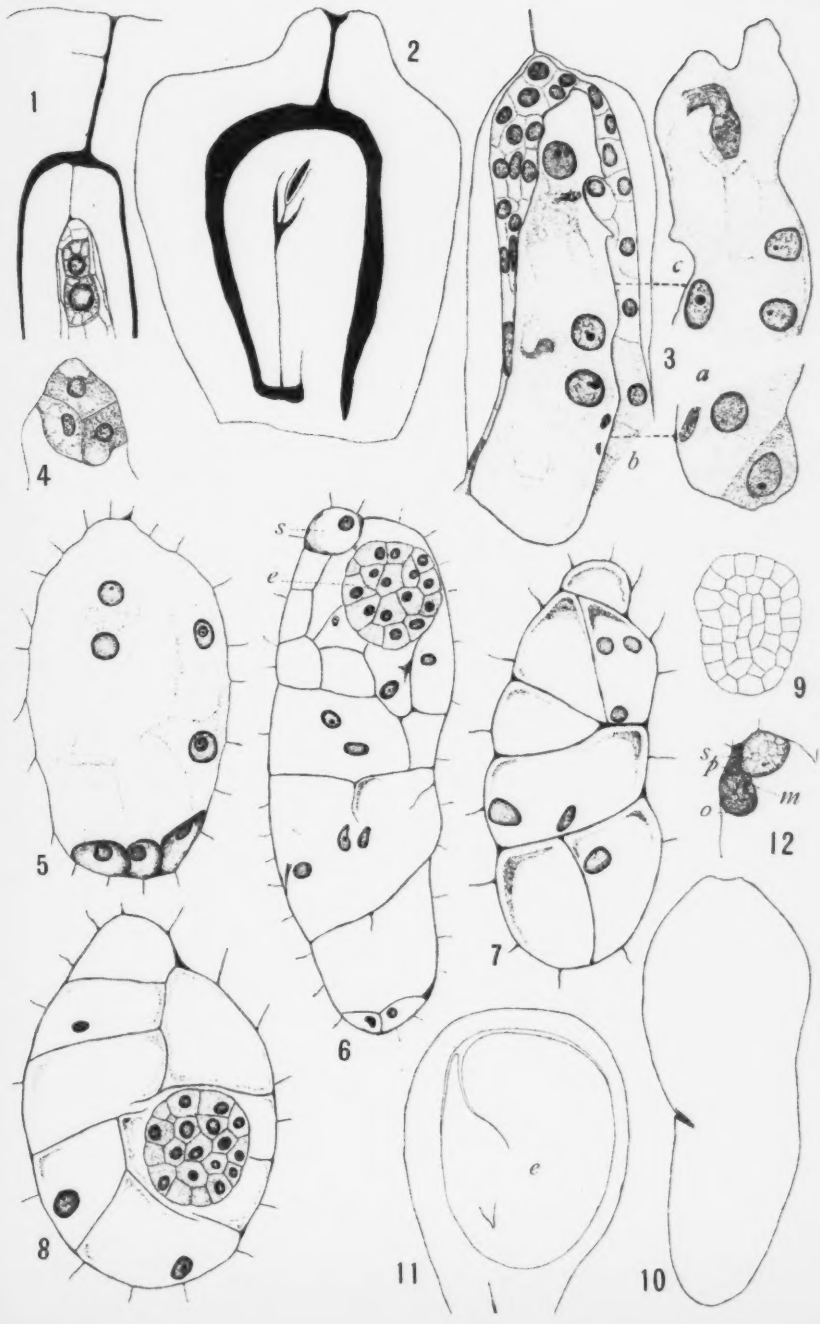
FIG. 38.—Eight-nucleate sac and two male cells (one fusing with a cell).

FIG. 39.—Fusion of polar nuclei.

FIG. 40.—Older sac, showing endosperm (*n*) and antipodals (*a*).

FIG. 41.—Sac showing endosperm (*s*) and proembryo (*e*).

FIG. 42.—Diagram showing later development of embryo (*n*) and endosperm (*e*).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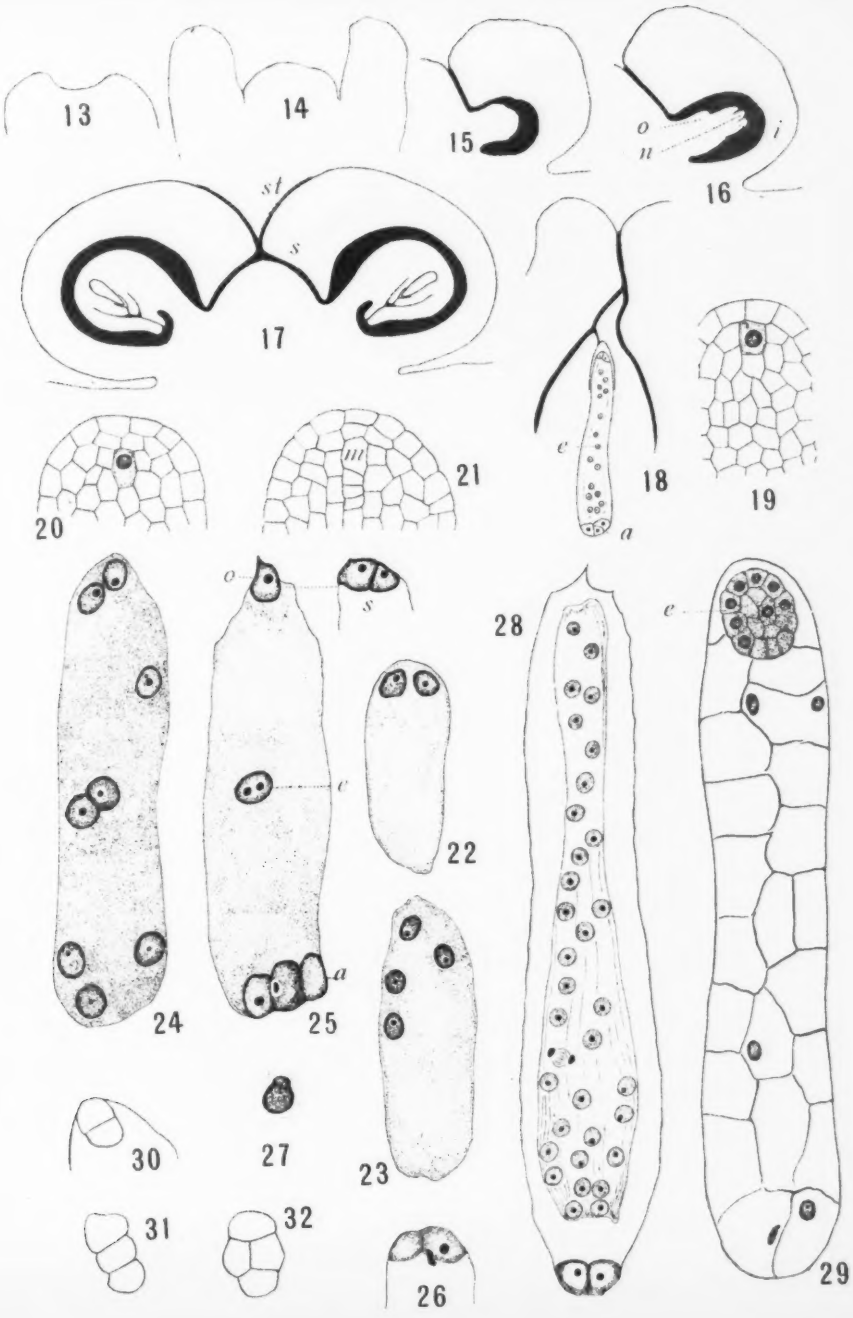
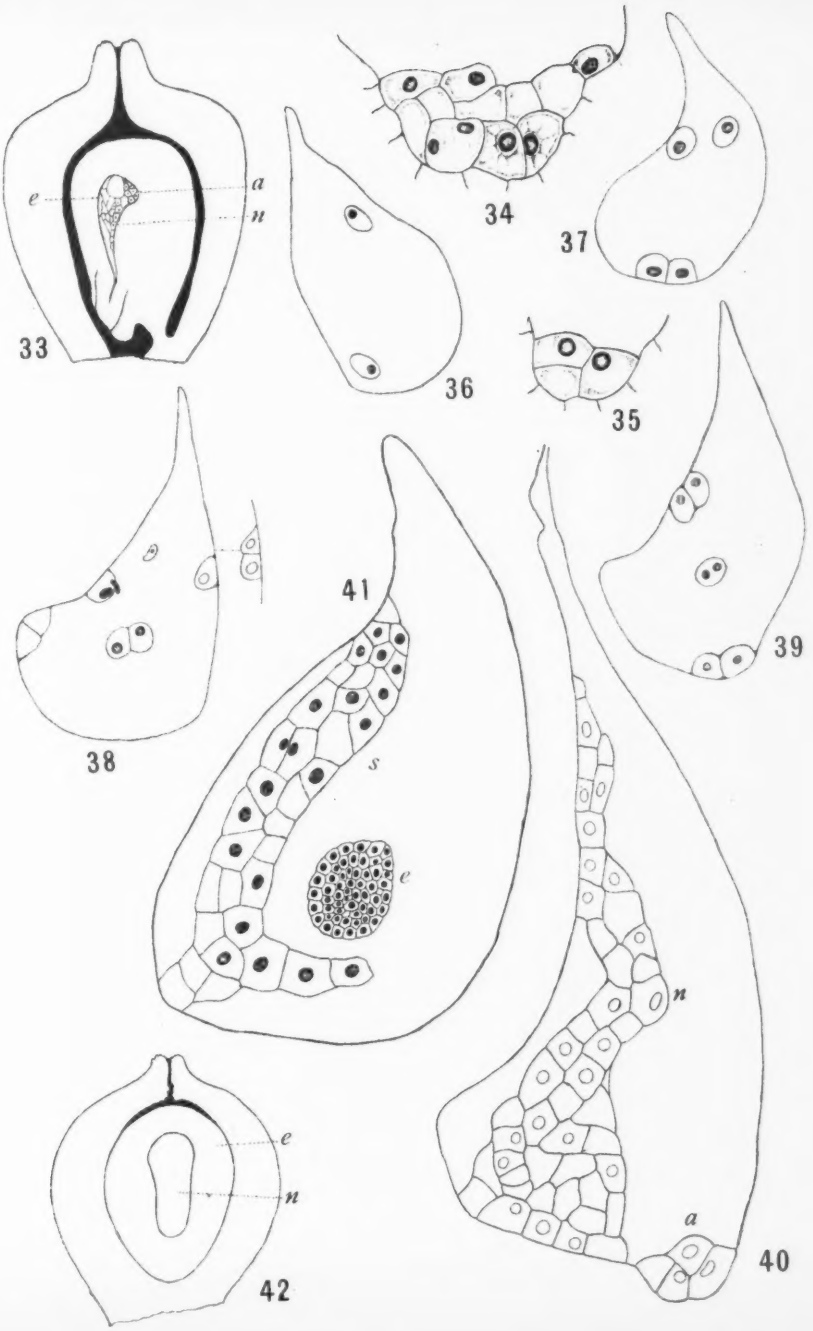


GOW on ARACEAE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THE EMBRYO SAC AND EMBRYO OF *GNETUM GNEMON*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 112

JOHN M. COULTER

(WITH PLATE VII)

Gnetum Gnemon has been made conspicuous among the other species of Gnetum chiefly by the investigation of LOTSY published in 1899.[1] The structures described were of such interest that it seemed desirable to supplement the somewhat incomplete account by a further examination. Accordingly material was obtained from the Philippine Islands and from British Guiana; the former from Dr. H. N. WHITFORD, and the latter from Mr. A. W. BARTLETT, director of the Botanic Garden at Georgetown. This material was first assembled by Dr. W. J. G. LAND, of this laboratory, in connection with his investigation of Ephedra; and he has kindly turned it over to me for separate study. The preparations and drawings were made by Dr. SHIGÉO YAMANOUCHI, of this laboratory, and to his technical skill the results are largely due.

The material included stages from two successive seasons, but unfortunately many intervening stages were not represented, so that no continuous account can be given. However, certain facts have been discovered that supplement and correct the previous accounts.

EMBRYO SAC

LOTSY described the embryo sac of *G. Gnemon* as showing an interesting deviation from those found by KARSTEN[2] in other species of Gnetum. Instead of containing only free nuclei at the fertilization stage, the embryo sac of *G. Gnemon* was described as containing

[1] LOTSY, J. P., Contributions to the life history of the genus Gnetum. Ann. Jard. Bot. Buitenzorg II. **1**:46–114. *pls. 2–11.* 1899.

[2] KARSTEN, H., Bot. Zeit. **50**:205–215, 221–231, 237–246. *pls. 5, 6.* 1892; Ann. Jard. Bot. Buitenzorg **11**:195–218. *pls. 17–19.* 1893; Cohn's Beitr. Biol. Pflanzen **6**: 337–382. *pls. 8–11.* 1893.

a compact antipodal tissue, sharply distinct from the micropylar chamber with its free nuclei. As a consequence, the embryo sac of *G. Gnemon* has been used ever since as illustrating a female gametophyte intermediate in structure between the tissue-filled sacs of Ephedra and Tumboa on the one hand, and the sacs of other species of Gnetum, which contain only free nuclei. Later the same investigator in reporting parthenogenesis in *G. Ula*[3] described the embryo sac of that species as being of the *G. Gnemon* type.

Our material of *G. Gnemon* does not confirm this account. At an early stage of the embryo sac, eight nuclei are observed grouped near the center (*fig. 1*), the sac being invested by the loose tissue of the nucellus. At a somewhat later stage the nucellar cells at the chalazal end of the sac are strikingly differentiated (*fig. 2*), becoming more and more compactly arranged, gradually obliterating the intercellular spaces, and taking on the appearance of glandular cells. The relation of this tissue in its early stage to the embryo sac is shown in *fig. 2a*. As vacuolation proceeds in the sac and the free nuclei become parietally placed, this "pavement tissue" becomes more compact and extends deeper into the chalaza (*figs. 3, 3a*). Still later it spreads laterally below, until it becomes fan-shaped in section (*figs. 4, 4a*), but it is always very distinct in contour and sharply marked off from the surrounding nucellar tissue. At the fertilization stage (*figs. 4, 5*) the sac contains only free nuclei, which become somewhat grouped at the antipodal end (*fig. 5*), but there is no walled tissue. Spreading below the sac, however, the mass of nucellar pavement tissue shows a definite contour, which might be merged in imagination with that of the sac and thus mistaken for a compact tissue within the antipodal end of the sac. LOTSY'S figures show the real contour of the sac, and his antipodal tissue is clearly this glandular pavement tissue developed in the chalaza. So far as the sac of *G. Gnemon* is concerned, therefore, its fertilization stage is that described for other species of Gnetum. It will be noted that after the fertilization stage is reached (*fig. 5*) the pavement tissue begins to lose its glandular character; and later it is destroyed entirely by the growing endosperm.

[3] LOTSY, J. P., Parthenogenesis bei *Gnetum Ula* Brongn. Flora 92:397–404. *pls. 9, 10.* 1903.

ENDOSPERM

A year later, the endosperm has destroyed all of the nucellar tissue except a very small amount at the tip (*figs. 6, 6a, 7a*), and it is clearly differentiated into a central region of smaller, more compact cells, and a more extensive peripheral region of larger and looser cells. In destroying the nucellar beak, a curious result is observed. The central region of the endosperm advances into the beak and then spreads laterally (*fig. 6*). In the meantime the peripheral region advances more slowly toward the beak, and as a consequence a ring of nucellar tissue is pinched between two growing masses of endosperm. The growth of the endosperm into the chalazal region also results in pressure toward the beak, so that the pinched nucellar tissue is under considerable pressure and becomes completely disorganized. Under this pressure some of the adjacent endosperm cells also become disorganized.

In ovules of the preceding year, at the fertilization stage of the embryo sac, a curious disorganization of some of the cells of the nucellar beak was observed (*fig. 5a*). Two transverse rings of cells, several layers beneath the epidermis, begin to disorganize; later the epidermis becomes involuted between the disorganized rings, resulting in a deep groove around the nucellus. The epidermal cells remain very vigorous in appearance, and when the endosperm develops into this region the groove disappears. The cause and the significance of this disorganization and of the temporary involution of the epidermis cannot be suggested.

EMBRYO

LOTSY has described the entrance of pollen tubes into the embryo sac, the fertilization of the free eggs, the excessive elongation of the fertilized eggs to form suspensors, and the cutting-off of the embryonal cells at the tip of the suspensor. Later stages in the development of the embryo have been described by BOWER,[4] but the intermediate stages have not been observed. Fortunately our material from the Philippines contained them, and revealed an unexpected situation.

When the endosperm has become fully developed, its peripheral

[4] BOWER, F. O., The germination and embryology of *Gnetum Gnemon*. Quart. Jour. Micr. Sci. **22**:278–298. *pl. 25*. 1882.

region contains a tangle of long, tortuous, and branching suspensors (*figs. 6, 7*), which are difficult to trace. During the formation of a suspensor by a fertilized egg, free nuclear division occurs, resulting in a few nuclei (four in *fig. 7*) distributed along the suspensor. Usually between these nuclei transverse walls are formed by the development of a cleavage plate from the wall of the suspensor. A cell at the tip of the suspensor is cut off in the same way, and contains one of the free nuclei, which becomes associated with numerous starch grains (*fig. 7*).

In this terminal embryonal cell free nuclear division continues (*figs. 8, 9, 10*), accompanied by cleavage walls, until a multicellular embryo is formed. In *figs. 9* and *10* it will be observed that this cleavage apparently continues until uninucleate cells are produced; and in our material this stage is reached first by a group of cells on one side of the embryo. It could not be determined whether this group holds any relation to a body region or not.

It has been supposed that in the embryogeny of Gnetum the preliminary stage of free nuclear division, common to other gymnosperms, had been eliminated; and that the first nuclear division was accompanied by wall formation, as in angiosperms. In *Gnetum Gnemon*, however, free nuclear division not only characterizes the proembryo, but also the early stages of the embryo. The case may be compared to that of Ephedra,[5] in which free nuclear division within the fertilized egg results in eight independent proembryonal cells, each of which continues free nuclear division and develops as a suspensor, which by a cleavage wall cuts off the terminal embryonal cell. In Gnetum the suspensor is formed by the fertilized egg instead of by a proembryonal cell, but the number of free nuclei formed by the egg in each case is approximately the same.

INTEGUMENTS

The mature seed of *Gnetum Gnemon* gives an opportunity to compare the integument and testa with those of other gymnosperms. *Fig. 6a* shows the seed slightly stalked within the so-called "perianth," which is fleshy. Two integuments are evident, and they develop in

[5] LAND, W. J. G., Fertilization and embryogeny in *Ephedra trifurca*. BOT. GAZETTE **44**:273–292. *pls. 20–22*. 1907.

basipetal succession. The inner one extends above to form the elongated micropylar tube, and at the maturity of the seed completely invests the nucellus (at this time replaced by the endosperm) as a papery layer. The outer integument becomes differentiated into an outer fleshy layer (white in the figure) and an inner stony layer (black in the figure), the latter completely investing the seed, the former being chiefly developed in the region of the nucellar beak. Two sets of vascular strands are present, the outer set traversing the fleshy layer of the outer integument, the inner set traversing the inner integument.

In Cycadophytes, Ginkgoales, and Coniferales, a single integument becomes differentiated into a testa of three layers: outer fleshy, stony, and inner fleshy. In Gnetum the same three layers are present, but the inner fleshy one has become differentiated in ontogeny as a separate integument. In all cases, this innermost layer finally forms a papery lining of the stony layer. Among the Pinaceae the outer fleshy layer is present in the integument, but it does not develop into the extensive pulpy investment that characterizes the Cycadales, Ginkgoales, and Taxaceae, a fact which is probably associated with the close investment of the seeds by the scales.

The variation in the distribution of the vascular strands among these layers is interesting. Among the more primitive Cycadofilicales and Cordaitales, in which the nucellus is relatively free from the integument, the outer set of strands traverses the outer fleshy layer and the inner traverses the peripheral tissue of the nucellus. In other Cycadofilicales and Cordaitales, however, and in Cycadales in which the nucellus and integument are free only in the region of the nucellar beak, the inner set of vascular strands traverses the inner fleshy layer of the integument; and this is the condition in Gnetum, except that this layer has become differentiated as an inner integument. In Ginkgoales the outer set of strands (belonging to the outer fleshy layer) is suppressed; in Taxaceae the inner set (belonging to the inner fleshy layer) is suppressed; and in Pinaceae both are suppressed.

MALE GAMETOPHYTE

It was a disappointment that the development of the male gametophyte was not secured, for it is only known among Gnetales in Ephe-

dra, in which it has been described by LAND.[6] The development of the tetrad was observed; and although the early anaphase of the first mitosis was not available for the counting of chromosomes, the late prophase of this mitosis and the anaphase of the second mitosis showed clearly that the chromosome numbers are 12 and 24.

SUMMARY

1. The "antipodal tissue" described by LOTSY as occurring in *Gnetum Gnemon* at the fertilization stage is a sharply differentiated nutritive tissue developed in the nucellus beneath the embryo sac, which at this stage contains only free nuclei, as described for other species of Gnetum.

2. Embryo formation begins with an excessive, suspensor-like elongation of the fertilized egg, accompanied by free nuclear division and cleavage walls; and the continuation of free nuclear divisions and cleavage walls in the embryonal cell until a multicellular embryo is formed.

3. The endosperm encroaches upon the tissue of the nucellar beak with some irregularity, an irregularity which reaches its extreme expression in Torreya, with its so-called "ruminated" seeds.

4. The inner integument of the ovule is the morphological equivalent of the "inner fleshy layer" of the single integument of other gymnosperms; and the occurrence of two sets of vascular strands is a relatively primitive condition, which has been departed from by Ginkgoales and Coniferales.

5. The chromosome numbers are 12 and 24.

THE UNIVERSITY OF CHICAGO

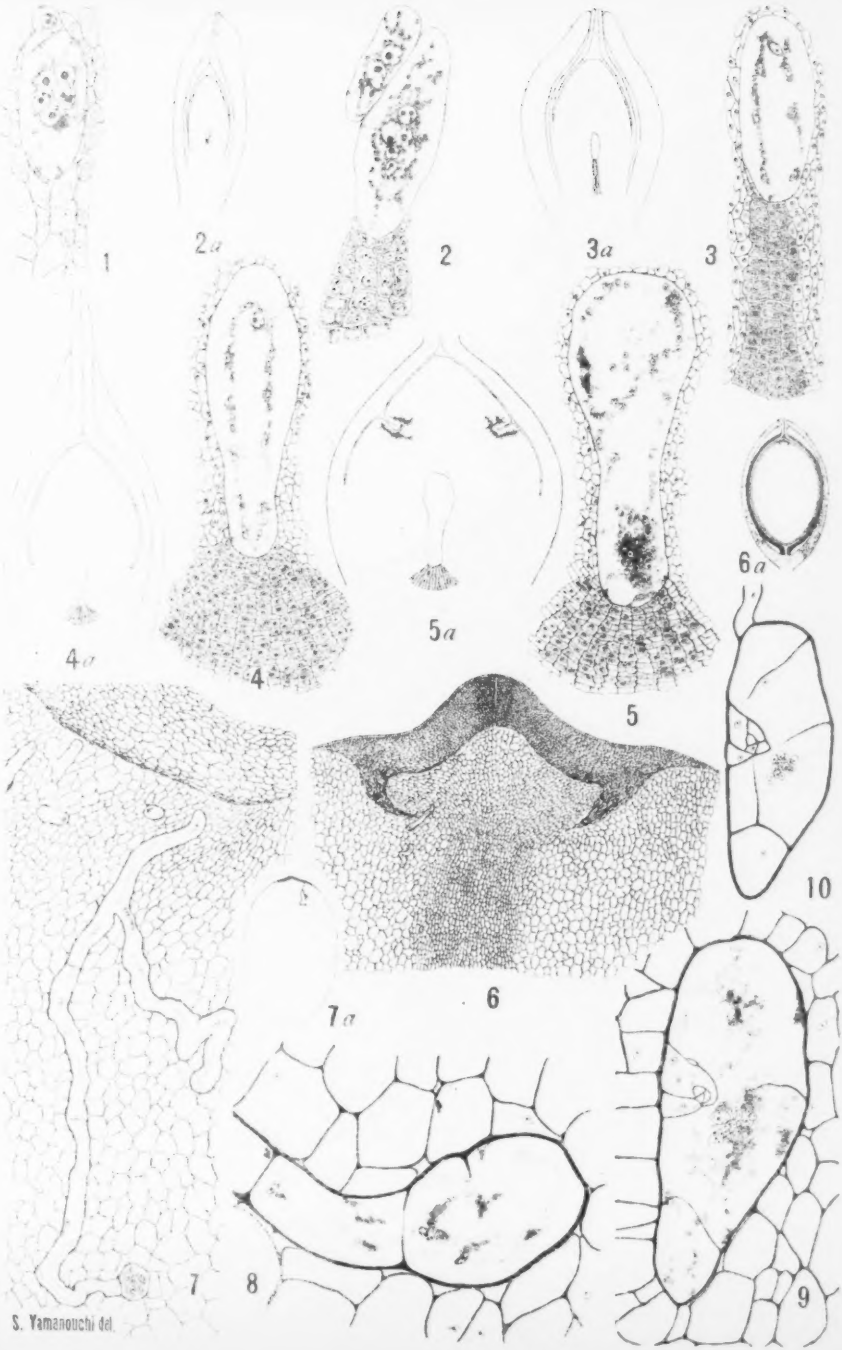
EXPLANATION OF PLATE VII

FIG. 1.—Embryo sac at an early stage, with centrally placed group of eight free nuclei; the cell above, with a large nucleus, is another embryo sac. ×500.

FIG. 2.—Somewhat later stage of embryo sac (all the nuclei not included), showing the beginning of the formation of the pavement tissue; a second embryo sac is also shown. ×500.

FIG. 2*a*.—An ovule at an early stage, showing the two integuments and the relation of the pavement tissue and embryo sac to the nucellus at the stage shown in *fig. 2*. ×40.

[6] LAND, W. J. G., Spermatogenesis and oogenesis in *Ephedra trifurca*. BOT. GAZETTE 38:1–18. *pls. 1–5*. 1904.



COULTER on GNETUM

FIG. 3.—Further development of pavement tissue; vacuolation of the sac. ×250.

FIG. 3*a*.—An ovule, showing general relation of regions shown in *fig. 3;* the tip of the nucellus has broken down (the only indication of a pollen chamber). ×40.

FIG. 4.—Further development of pavement tissue and embryo sac (probably fertilization stage). ×220.

FIG. 4*a*.—An ovule, showing relation of regions shown in *fig. 4*. ×40.

FIG. 5.—Later stage of embryo sac (possibly still in fertilization stage), with grouping of nuclei in the antipodal region, where tissue formation probably begins; beginning of disorganization of pavement tissue. ×210.

FIG. 5*a*.—An ovule, showing relation of regions shown in *fig. 5;* also the curious disorganization of cells and infolding of the epidermis in the nucellar beak. ×40.

FIG. 6.—Tip of seed; small amount of tissue of nucellar beak not destroyed by endosperm; differentiation of central and peripheral regions of endosperm, the former having advanced into the center of the nucellar beak and spread laterally, resulting in crushing nucellar tissue against the encroaching peripheral region of endosperm; sections of two suspensors shown. ×40.

FIG. 6*a*.—Mature seed invested by the fleshy "perianth;" outer integument differentiated into outer fleshy (white) and stony (black) layers; inner integument forming the micropylar tube; at apex of nucellus is indicated the remains of the nucellar tissue (shown with greater magnification in *fig. 6*). ×2.

FIG. 7.—A proembryo, showing the branching, suspensor-like elongation of the egg, with free nuclei and cleavage walls; also the embryonal cell containing a nucleus and numerous starch grains; sections of other suspensors shown, and also a small portion of the undestroyed tip of the nucellus. ×40.

FIG. 7*a*.—Outline to show the relation of parts illustrated by *fig. 7*. ×3.

FIG. 8.—Beginning of embryo formation by the embryonal cell, showing free nuclei and the beginning of a cleavage wall; a cleavage wall in the suspensor also shown. ×250.

FIG. 9.—Section of later stage of embryo, showing free nuclei, cleavage walls, and the beginning of small-celled tissue at one side. ×210.

FIG. 10.—An embryo reconstructed from serial sections, showing free nuclei, cleavage walls, and the beginning of uninucleate cells. ×140.

BRIEFER ARTICLES

THE OCCURRENCE AND RATE OF PROTOPLASMIC STREAMING IN GREENHOUSE PLANTS

In the Botanical Laboratory of Smith College, under the direction of Professor GANONG, various lines of investigation are in progress to determine which plants of those available during the school and college year are best adapted for educational work in each of the principal physiological processes. The results are appearing from time to time in the BOTANICAL GAZETTE.[1] The object of the present inquiry is to discover which of such plants show protoplasmic movement, in which the streaming is most active, and at what temperatures.

The development and sum of our knowledge of protoplasmic streaming may be traced through PFEFFER'S *Plant physiology*, which needs to be supplemented, however, by the references under "Protoplasmabewegung" in JUST'S *Jahresbericht*, and by the admirable new work of EWART, *On the physics and physiology of protoplasmic streaming in plants* (Oxford, Clarendon Press, 1903). Streaming has been found in a great number of plants of the most diverse groups, from fungi to phanerogams, and in the most different structures, including the exposed cells of algae (where it reaches its finest display), emergences and hairs on various organs (notably stamens), root hairs (where it has been found in at least sixty-five families), plasmodia of myxomycetes, mycelia of molds, pollen tubes, the bark, young wood, and medullary rays of various trees, stamens, petals, and other parts. The rate of the streaming has been measured by several observers in different plants, and ranges from zero up to an extreme of 10^{mm} per minute, but in each plant the rate is dependent upon temperature and rises from a minimum of no movement up to an optimum of greatest movement, whence it sinks to a maximum of no movement, which cardinal points vary with the species but approximate in a general way to 5°, 35°, and 45°. The significance of the streaming is not known, and the explanatory guesses range all the way from "pathological," through "incidental," to "ecological," the most reasonable of the latter (especially in view of its greater activity in large cells) being that it is a mechanical aid to diffusion.

Protoplasmic streaming is a striking and perhaps a fundamentally important phenomenon, well worth demonstration to elementary classes

[1] **40**:302. 1905; **45**:30. 1908; and **45**:254. 1908.

and measurement by advanced ones. Of the plants available for the purpose in winter, namely, those which are or may readily be grown or kept in greenhouses or houses, the best known heretofore are as follows: species of Chara and Nitella, which may be kept over winter in tubs under the greenhouse benches; Elodea and Vallisneria, responsive to the same treatment; *Tradescantia zebrina* or wandering Jew, very commonly grown, and *T. virginica* (or *pilosa*) which can be kept part of the winter if planted on a greenhouse bench and cut back until July or August; squash or tomato, which can be grown from seed in two or three weeks; root hairs of mustard and other plants, which can be grown to perfection in wet, covered flower-pot saucers; heliotrope and pelargonium.

The brevity of this list in plants presenting material ready at all times led me to undertake a systematic search for others through a range of educational greenhouses, and I was successful in finding the considerable number of new cases marked by the asterisks in the table below.[2] I also undertook to determine, upon a uniform system, the rate of movement, both for these and for the other common forms, at the two temperatures practically most important, namely 20° (approximately ordinary room temperature) and the optimum; and the results are recorded in the table. For the control of temperature I used GANONG'S temperature stage, with the method, including standardized ocular micrometer and metronome, described in his *Laboratory course in plant physiology*. The figures in the table are averages of two or more determinations from different specimens except in a few cases of the optimum, where only one measurement was made.

GREENHOUSE PLANTS SHOWING PROTOPLASMIC STREAMING, WITH ITS RATE

NAME OF PLANT	PARTS OBSERVED	RATE (mm. per min.)	
		@ 20°	Optimum
*Abutilon striatum hyb.	unicellular hair from surface of ovary	.161	.773@32°
	hair from calyx	.016	
*Ampelopsis Veitchii (Boston ivy)	unicellular hair from under side of midrib	.138	.368@36°
Avena sativa (oat)	root hair of seedling	.322	
*Azalea sinensis	cell of hair from leaf blade	.138	.322@34°
Brassica alba (white mustard)	root hair	.322	

*Those marked by an asterisk are here recorded for the first time.

[2] As concerns outdoor plants for this same purpose there is a paper entitled "Subjects for protoplasmic movements," by B. D. HALSTED, in Bull. Bot. Depart. Iowa State Agric. Coll. **1888**:578. It enumerates ten common plants.

Name of Plant	Parts observed	Rate (mm. per min.)	
		@ 20°	Optimum
*Campanula glomerata (bell flower)	unicellular hair from leaf blade or petiole	.258	.644@38°
*Capsella Bursa-pastoris (shepherd's purse)	unicellular hair from flower stalk	.215	.483@32°
*Cestrum elegans	unicellular hair from stem or veins on under side of leaf	.161	.428@35°
*Coleus Blumei Verschaffeltii	cell of hair from stem at node or edge of petiole	.276	.892@35°
Cucurbita maxima (squash)	cell of hair from petiole	.258	.859@32°
*Cuphea hyssopifolia	cell of hair from stem	.121	.258@34°
*Cuphea ignea (cigar plant)	cell of hair from stem	.048	.215@36°
Elodea canadensis	cell near midrib of leaf	.604	.966@36°
*Fuchsia speciosa	unicellular hair from flower stalk	.175	.322@34°
*Gaillardia grandiflora	cell of hair from petiole	.227	.703@36°
*Gloxinia speciosa	cell of hair from stem or petiole	.322	1.054@36°
	cell of hair from leaf blade	.351	1.088@36°
*Helianthus annuus (common sunflower)	cell of hair from edge of petiole	.175	.429@35°
	cell of hair from midrib	.081	
Heliotropium peruvianum (common heliotrope)	unicellular hair from stem, surface of leaf, or under side of midrib	.121	.386@34°
*Hibiscus Cooperi (rose mallow)	unicellular hair from petiole or under side of midrib	.175	.773@35°
*Isoloma hirsutum	cell of hair from stem	.193	.515@34°
*Lavandula vera (lavender)	cell of hair from stem	.242	.508@35°
*Lobelia Erinus	unicellular hair from leaf edge, petiole, or stem	.297	1.073@37°
*Lopezia albiflora	unicellular hair from stem	.193	.483@30°
	unicellular hair from leaf	.322	.858@30°
	epidermal cell from under side of midrib	.121	.322@32°
Nitella (common species)	young cells	1.610	2.840@34°
Pelargonium quercifolium (oakleaf pelargonium)	cell of conical hair from petiole or surface of leaf	.322	.552@33°
Lycopersicum esculentum (common tomato)	cell of hair from stem, leaf edge, or vein	.386	1.288@34°
*Oxalis Bowiei	cell of hair from leaf-edge	.242	.575@34°
*Primula obconica (primrose)	cell of hair from stem or leaf	.276	.805@30°
*Saxifraga cotyledon palmata (saxifrage, rockfoil)	cell of hair from edge of leaf	.176	.859@37°
*Saxifraga sarmentosa (beefsteak saxifrage)	cell of hair from flower stalk	.322	.859@35°
*Senecio cruentus (cineraria)	cell of hair from under surface of leaf	.193	.386@31°
*Streptosolen Jamesonii	cell of hair from stem or leaf blade	.183	.428@37°
Tradescantia virginica (spiderwort)	cell of stamen hair	.242	.644@33°
Tradescantia zebrina (wandering Jew)	cell of stamen hair	.386	.990@32°
*Vinca major (periwinkle)	cell of hair from leaf edge	.107	.322@35°
*Whitlavia grandiflora	unicellular hair from petiole or calyx	.242	.644@38°
	" " " leaf blade	.193	
	cell of glandular hair from leaf blade	.138	

* Those marked by an asterisk are here recorded for the first time.

This table shows that suitable material can easily be obtained in winter for the demonstration of protoplasmic streaming. Of the plants studied, I should recommend Nitella and Elodea as good examples of rotation; *Gloxinia speciosa*, *Tradescantia zebrina* and *virginica*, *Abutilon striatum* (hyb.), *Lycopersicum esculentum*, *Whitlavia grandiflora*, *Cucurbita maxima*, *Lobelia Erinus*, and *Saxifraga cotyledon palmata* for circulation. *Gloxinia speciosa* is especially good, as the entire hair cell can be brought within the range of vision, and a constant change observed in the arrangement of the strands, circulation passing into rotation as the temperature rises. Campanula, Lobelia, Vinca, Streptosolen, Capsella, and Ampelopsis have markings on the cell wall which obscure the view of the protoplasm to some extent.

A complete study of the streaming involves the measurement of its rate at various temperatures from minimum through optimum to maximum. Results of such study, expressed in a graph, are available for Chara, Elodea, and Vallisneria in DAVENPORT'S *Experimental morphology* (1:226), and for a common Nitella in GANONG'S *Laboratory course in plant physiology* (p. 19). My own graph for Tradescantia shows a curve much flatter than that above mentioned for Nitella, though otherwise somewhat similar to it.—GRACE L. BUSHEE, *Smith College, Northampton, Mass.*

ON PLASMOLYSIS

According to the conception of plasmolysis developed by DE VRIES and PFEFFER, the contents of a cell contract and round up when it is placed in a solution whose osmotic pressure exceeds that of the cell sap. This is explained by supposing the outer layers of protoplasm to be impermeable to the substances in solution which produce the osmotic pressure. If the protoplasm is permeable to these substances, either wholly or in part, deviations from the rule given above will occur. Deviations have in fact been described by several authors and explained by supposing the protoplasm to be more or less permeable to the substances in solution, which enter the cell sap and increase its osmotic pressure.

I propose in this paper to describe deviations which range from those which are very slight to those which are of extraordinary intensity, the explanation of whose nature is entirely different from the one mentioned above.

My attention was first called to these deviations some three years ago by the results of some of my experiments on the rôle of osmotic pressure[1]

[1] A brief account of these investigations appeared in Univ. Calif. Publ. Bot. 2:227, 229. 1906.

in marine plants. I then experimented on fresh-water plants and found even more striking results.

In order to make clear the nature of these results I will describe an experiment with Vaucheria. Zoospores which had attached themselves to slides were allowed to germinate and produce short tubes. The slides were then transferred to 0.0937 *m* NaCl solution. In the course of a few minutes the protoplasm began to contract away from the cell wall. The solution was apparently strong enough to produce plasmolysis and I prepared weaker ones. These, however, produced the same effect, taking more time to do so in proportion as they were weaker. It then occurred to me that the effect was not due to osmotic pressure but to a *contraction of the protoplasm due to the chemical action of the salt.*

In order to test this idea I endeavored to determine how dilute the solution could be made and still produce this effect. I found that even 0.0001 *m* solution produced it, though usually only after a day or so. The experiments are repeated several times with Kahlbaum's C. P. sodium chloride which I had recrystallized six times. The result remained the same.[2]

I then tried to do away with this effect. This is easily accomplished by adding a little $CaCl_2$. The addition of $CaCl_2$ in solid form increases the osmotic pressure of the solution, but in spite of this it prevents the contraction of the protoplasm away from the cell wall. If one molecule of $CaCl_2$ is present for every hundred molecules of NaCl, the algae endure solutions of 0.1 *m* (that is to say, solutions with a thousand times greater osmotic pressure) without any contraction or apparent plasmolysis.

I have since experimented with a great variety of salts and combinations of salts which produce effects similar to those just described. I have found that all the plants with which I have experimented (algae, fungi, mosses, liverworts, Equisetum, flowering plants) give similar results, though most of them are much less sensitive than Vaucheria.

After these experiments I was in no way surprised when I found that water distilled from a metal still could produce apparent plasmolysis within a few minutes, and that this could be prevented by the addition of various substances.

In what way is the contraction just described distinguishable from true plasmolysis? In many cases it cannot be so distinguished at all[3] by its

[2] Vaucheria from other localities and especially in later stages proved less sensitive to the action of NaCl. Cf. Jour. Biol. Chem. 1:363. 1906.

[3] It is possible that the results described by DUGGAR (Trans. St. Louis Acad. Sci. 16:473. 1906, and TAKEUCHI (Bull. Coll. Agri. Imp. Tokyo Univ. 7:623. 1908) are explicable on this basis.

appearance, but only by comparison with other (especially non-toxic or balanced) solutions, or by determination of the freezing-point of the cell sap. The protoplasm rounds up just as in true plasmolysis, and may even subsequently recover and expand in characteristic fashion when transferred to distilled water.

It may happen that a contraction is caused by true plasmolysis, but the subsequent chemical action of the salt renders the protoplasm unable to recover and expand when the cell is transferred to distilled water. Such recovery cannot therefore be used as a means of distinguishing true plasmolysis from a contraction due to chemical action.[4] In some cases the contraction may be distinguished from true plasmolysis by the irregular outline of the contracted protoplasm.

On looking over the literature it becomes evident that such precautions as are necessary to distinguish this contraction from true plasmolysis have not been generally observed in making plasmolytic determinations, and a revision of such determinations is necessary. Determination of the freezing-point of the cell sap will certainly be needed in many cases if an accurate result is desired.

In conclusion I would call attention to the importance of this discovery in respect to one of the most prominent biological problems, the question whether salts are able to penetrate the protoplasm or not. Far-reaching conclusions have been drawn from the fact that when a cell is placed in a solution of a certain substance the protoplasm contracts and does not subsequently expand if left in contact with the solution. This has been interpreted to mean that the substance in solution is unable to penetrate the protoplasm. I find, however, in many cases, that the true interpretation is exactly the opposite. The permanent contraction of the protoplasm is caused by the *penetration of the substance in question* which produces chemical effects upon the protoplasm, wholly different from those produced by a substance whose action is purely osmotic.

In view of this we cannot give credence to certain very important conclusions and theoretical considerations which have been based on this criterion of penetrability. These points will receive fuller discussion in a subsequent paper.—W. J. V. Osterhout, *University of California.*

[4] The expression chemical action is here used in a very broad sense to include effects which are not osmotic in character.

CURRENT LITERATURE

BOOK REVIEWS

The origin of a land flora

Under this title Professor BOWER has written a volume[1] which must be regarded as the culmination of his important studies during the last twenty years. It is a formal and amplified statement of the views advanced in his series of five papers entitled "Studies in the morphology of spore-producing members," published between 1894 and 1903.

The scope of the volume is very broad and its spirit is admirable. The author recognizes that his thesis is not proved; that in the very nature of the case it never can be; and that there are many possible alternatives. However, he presents so strong a case that the truth of the theory would not come to anyone as a surprise. Naturally the book is speculative, and the author's frequent insistence that this is the case should be respected by those who follow its doctrine. The human mind seems to be so constituted that when a view is distinctly formulated it thereby seems to gain additional proof.

There is a strong tincture of teleology at every turn, the elimination of which would have strengthened the discussion. This gives it a flavor of unreal "other-worldliness" that is becoming unscientific. That a certain structure would be useful to a plant and therefore it grows, is hardly acceptable as an explanation of origins.

The thesis of the volume is the origin of the sporophyte as an antithetic generation, which has become fixed and amplified by the invasion of the land. In developing this doctrine, the author presents first a statement of the working hypothesis (pp. 254), then a detailed statement of facts (pp. 403), and finally the general comparisons and conclusions (pp. 60). In a brief review it is impossible to present the suggestions that fill these three parts. The second part—the statement of facts—is wisely separated from the other two, and in this form it represents an admirable treatment of the morphology of bryophytes and pteridophytes, especially the latter. Much new material is included here, and especially helpful is the massing of the morphological, anatomical, and paleobotanical evidence. These three points of view are too often kept separate, when they should serve as checks upon one another.

The two theoretical parts also contain much more detail than can be presented. However, the broad outlines of the working hypothesis and some of the more important conclusions may be indicated.

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[1] BOWER, F. O., The origin of a land flora: a theory based upon the facts of alternation. 8vo. pp. xii+727. *figs. 361.* London: Macmillan and Co. 1908. $5.50.

Running throughout the hypothesis is the assumption that the most critical and hence controlling fact in the life of any organism is the relation existing between environment and fertilization. Perhaps the most fundamental conception is that the "Archegoniatae" are amphibious. (It may not be out of place here to suggest that the group name "Archegoniatae" has about outlived its usefulness. To associate the widely separated bryophytes and pteridophytes in this way, and to include or exclude part of the gymnosperms, is a grouping too unnatural and misleading to be continued.) That the gametophyte of "Archegoniatae" is amphibious means that it is just as aquatic as an alga, and shows this in its delicate structure, lack of intercellular spaces, lack of a water-conducting system, and the possibility of fertilization only in the presence of water. "The gametophyte proclaims its ultimate dependence on external fluid water as thoroughly as an alga." On the other hand, the sporophyte is characteristically an aerial body, with its more robust habit, "ventilating" system, vascular strands, and spores adapted for dryness.

Attention is called to the fact that the "Archegoniatae" retain with remarkable pertinacity this "awkward and embarrassing" method of fertilization; but that with the advent of the seed plants this becomes modified, and the higher seed plants at last become completely terrestrial.

A general outline of the steps by which the sporophyte was established and amplified as a terrestrial structure may be stated as follows: The gametophyte was the previously existing phase, and the initial step in the appearance of the sporophyte was the "post-sexual divisions, giving rise to a plurality of germs," such as is observed in the life-histories of certain green algae. In plants exposed to changing conditions of moisture and drought the fixing of such a generation would be assured, according to the following logic: external water would be occasional rather than constant; hence the sexual act would be occasional; hence there would be less dependence upon the sexual act for multiplying individuals; hence a "premium would be put upon" the other method of propagation suitable for drier conditions. Thus the sporophyte is to be recognized as a body originating as an adaptation to terrestrial life.

In amplifying the sporophyte, it is assumed that the first and also the final office of the sporophyte is to produce spores, and that the larger the number of spores (in homosporous forms) the better the chance of survival; therefore the increase in number of spores is "encouraged." But to protect spores when young and to nourish them during development presupposes some vegetative system.

The process for developing this vegetative system is the familiar theory of progressive sterilization, so well traced by the author among bryophytes. Among bryophytes, however, there are certain limits imposed by mechanical and physiological conditions; while among vascular plants there is greater freedom for progress, chiefly by the segregation of the sporogenous tissue into distinct sporangia and the formation of "appendicular organs." As a result of this progressive sterilization and organization of vegetative tissues, the fertile tissue appears later and

later in ontogeny, until what was first in phylogeny comes to be last in ontogeny. This disposition of the recapitulation theory appears in various places throughout the volume, and it is evident that the author regards it as reliable only within certain limits, when it agrees with other evidence.

From among the general conclusions the following may be selected as of most general interest. The author concludes that no definite algal form now living can be held to have been a direct progenitor of any known archegoniate type; but that certain algae suggest in their post-sexual phase how the initiation of the sporophyte may have occurred. Also the liverworts and mosses may be held to be "blind branches of descent;" but they illustrate changes that suggest the origin of sterile tissues from those potentially fertile, and the final establishment of a self-nourishing system in the sporophyte.

The gametophyte of early pteridophytes was probably a relatively massive green structure, with deeply sunk sexual organs; and the sporophyte was the strobiloid type of body illustrated by *Lycopodium Selago* and its allies (the author's well-known "theory of the strobilus"). This reconstruction of the most primitive vascular body is obtained by converging all the known pteridophyte lines, and the result is a body which arose from no one knows what, but which does not suggest any known bryophyte or alga.

This is a very meager outline of the contents of a large volume, crowded with facts and suggestions. Such speculation brings perspective and stimulus, and its only danger is a confusion of theory with fact, for which the author in this case could not be held responsible. Perhaps the most serious charge that could be brought against him, in this connection, is his great command of picturesque statements, which are highly figurative but contain perilous suggestions for the unwary. For example, that "encouragement was given" to a multiplication of spores; that the method of fertilization in archegoniates is "awkward and embarrassing;" that "a premium was put upon" aerial spores, etc., are extremely telling forms of statement, but they are more rhetorical than exact, suggesting far more than was intended.

At all events, the volume is a monument to the research power and philosophical insight of its distinguished author.—J. M. C.

Electro-physiology

Two years ago plant physiologists were rather taken aback by a large volume of researches on plant response from the pen of a contributor previously unknown in this field. It is not often that so brilliant a display marks the advent of a new scientific luminary, and it was impossible at once to determine whether he was a meteor, whose light would flash and disappear, or a star of the first magnitude. There were not wanting, indeed, signs that he was erratic. This work[2] conspicuously lacked relation to the present state of knowledge and showed scant acquaint-

[2] BOSE, J. C., Plant response as a means of physiological investigation. See review in BOTANICAL GAZETTE 42:148. 1906.

ance with the researches of others; while the naïveté with which some of the most recondite problems were attacked and dismissed as solved awakened amused incredulity. At the same time the author's ingenuity in devising and adapting self-registering methods to the mechanical and electrical responses of plants, as well as the suggestiveness of some of his results, made the book possibly an epochal one, since it struck out a rather new path for most important investigations.

So far, however, nothing has come of it. Its reception has been apathetic, not to say cold, and to all appearances it has fallen dead because of its faults and in spite of its manifest virtues. No researches have followed it up; no investigator has used its methods. That volume on *Plant response* was the second of a series of three (the first on *Response in the living and non-living*), of which the third has just made its appearance under the title *Comparative electro-physiology*.[3]

The author herein shows the same peculiarities as in the preceding volume. There is the same naïve interest in well-known phenomena, as though they were quite novel; there is the same lack of effort to connect his work with that of others, so far, at least, as citing their researches or results is concerned. It is doubtful if in this book of over 800 pages, dealing with a very special topic on which there must be a legion of workers, there are a dozen citations of original sources. There is much repetition of the earlier volume; the same tilting at windmills—vigorous attacks on vitalism and on the contrast between animals and plants, and between "sensitive plants" and others, till one is tempted to inquire with Sairey Gamp, "Who denyges of it, Betsey, who denyges of it?"

This volume maintains the same simple thesis as its predecessor—as simple as the faith of Islam, "Allah is great and Muhamed is his prophet"—contraction is universal and negativity is its sign. The direct response to every stimulus is "contraction," the indirect one (at a little distance) is "expansion." The former is said to be *accompanied by* reduction and the latter by increase of turgidity, with corresponding electric variations. The expression of contraction in movement, "suction" (ascent of sap), growth, "torsion" (in climbing plants), death, and electric variation, constitutes the theme of the two volumes. This much needs to be said: There are reasons for expecting some universal fundamental reaction in all responses; it may be that BOSE has hit upon it in what he miscalls "contraction" (for this is really nothing but a reduction in turgidity); but his work is not convincing.

This book shares with its predecessor, also, the confusion between energy and stimulus, a confusion that is possible because we know so little of plant energetics. This reaches its absurd climax in the conception of the author as to the function of plant nerve. He gravely tells us that the ramification of the "nerves" in a leaf provides a "virtual catchment basin for the reception of stimulus," whence it is transmitted to the body of the plant, there to be stored and

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[3] BOSE, J. C., Comparative electro-physiology, a physico-physiological study. 8vo. pp. xliv+760. *figs. 406*. New York: Longmans, Green & Co. 1908. $5.75.

used later in responses. All this is quite apart from the question of the energy fixed in photosynthesis.

This suggests another fault. BOSE seems to ignore, if he is not ignorant of, the anatomy of the parts with which he is dealing. Skin is skin, whether it be the skin of plant or animal. Stomach of Gecko and pitcher of Nepenthes (an open stomach of a primitive type, he calls it) are all one to him; they behave alike, in spite of their extraordinary differences of structure. Nerves of animal and nerves of plants, which have been identified by their behavior as the vascular bundles of plants, are physiologically the same. Digestion being a "diphasic process," that is, consisting of secretion first and absorption afterward, roots, which secrete substances that dissolve their mineral "food" and then absorb it, must be another kind of open stomach; and besides, they behave electrically as stomachs do! Now if these things are so, it argues that the responses with which BOSE is dealing are either extremely superficial or extremely fundamental, inhering in all living matter; and in either case they would be of little significance.

The situation seems to be most peculiar. BOSE, we judge, is a brilliant experimentalist, trained first as a physicist, but inadequately equipped with knowledge of what has been done in the field of plant irritability. This has been at once an advantage and a snare. It has left him free to present his researches untrammeled by the conventional view, but it has exposed him to mistakes which a little more knowledge would have avoided. It has given him courage to attack the most knotty problems, but it has led him to satisfaction with inadequate conclusions. Apparently, too, he has become possessed by a theory as to the nature of response, and under the yoke of that theory he makes all his captive facts to pass.

Out of these books we look for some one to rescue many good observations, now apparently gone awry; and by his methods and apparatus in the hands of real physiologists we hope soon to see made important advances in the knowledge of *Reizphysiologie*.—C. R. B.

Conference on Genetics

The report[4] of the Third International Conference on Genetics held under the auspices of the Royal Horticultural Society, July 30 to August 3, 1906, is a veritable treasure-trove for students of heredity, hybridization, and plant-breeding, both in their theoretical and practical aspects. Besides giving a minute account of the doings of the Conference, there is a brief but excellent sketch by the secretary, Rev. W. WILKS, of the life and work of GREGOR MENDEL, illustrated by three good portraits, a fine view of the Abbey at Brünn, and a facsimile letter written to NÄGELI. Excellent portraits are also given of some of the more

[4] Report of the Third International Conference, 1906, on Genetics; hybridization (the cross-breeding of genera or species), the cross-breeding of varieties, and general plant-breeding. Edited by the Rev. W. WILKS. 8vo. pp. 496. *figs. 131*. London. 1907.

prominent participants in the Conference, as LAWRENCE, WILKS, BATESON, JOHANNSEN, TSCHERMAK, WITTMACK, HURST, Miss SAUNDERS, and the VILMORIN brothers.

Besides the address of the chairman, Prof. BATESON, upon "The progress of genetic research," there are a number of important contributions to Mendelian inheritance, by HURST, DARBISHIRE, DAVENPORT, Miss SAUNDERS, TSCHERMAK, and BIFFEN; two papers upon orchid hybrids by ROLFE, and CRAWSHAY; and several papers upon the occurrence of natural hybrids, the most comprehensive of which by E. G. CAMUS is not printed in full because it was sufficiently voluminous to make a separate book. This paper deals with the spontaneous hybrids of the European flora. A lengthy list of natural hybrids is also given by LYNCH, Curator of the Botanic Garden, Cambridge. PFITZER deals briefly with hybridization and the systematic arrangement of orchids, and a valuable posthumous paper by the same author discusses the probability of the origin of the Orchidaceae from the Amaryllidaceae. OSTENFELD describes some castration experiments with Hieracium, and ROSENBERG reports briefly his work upon the cytology of Drosera, and also on Hieracium hybrids. In some of the latter he has found considerable differences in the number of chromosomes carried by different eggs of the same plant. BUNYARD reviews the question of xenia, stating that after an extensive search for evidence of this phenomenon he knows of but a single instance, this being an apple cross made by him ("Sandringham" × "Bismarck"). J. H. WILSON describes a considerable number of infertile hybrids, which he has studied with some care.

Dealing with plant breeding from the more practical point of view are the following: E. F. SMITH outlines the plant-breeding operations of the U. S. Department of Agriculture; the improvement of sugar cane is discussed at length by Sir DANIEL MORRIS; papers by ZAVITZ, P. DE VILMORIN, C. E. SAUNDERS, and BIFFEN describe results in breeding improved races of wheat and other small grains; HANSEN tells of his work in breeding cold-resistant fruits; RIVERS and LAXTON also give some of their results in hybridizing fruits, particularly peach × nectarines, apple hybrids, and plum hybrids; LAXTON draws some conclusions from the work with peas for which his family is justly famous; WARD gives a finely illustrated description of some of his excellent work on carnations, explaining among other things his method of keeping pedigree records; VAN TUBERGEN, of Haarlem, discusses hybrids among bulbous plants; PAUL gives the derivation of a number of fine hybrid roses of recent production; H. H. GROFF, the specialist in Gladiolus, discusses plant-breeding from the point of view gained by work with these plants, and emphasizes the idea that the plant-breeder has much to learn from the animal-breeder. There are several shorter papers by other authors.

Many of the articles are illustrated with fine halftones and the press work is up to the high standard for which the reports of the Royal Horticultural Society are well known.—GEORGE H. SHULL.

North American trees

Three years ago this journal[5] noticed the appearance of SARGENT's excellent *Manual of the trees of North America*, which brings into a convenient volume the information that is much more elaborated in his great *Silva*. Now another manual of the trees has appeared, bearing the title *North American trees*, and written by BRITTON and SHAFER.[6] The very handsome volume is made bulky by the heavy paper, so that it will have to be used more as a standard dictionary than as a handy manual.

The distinct mission of the volume, however, is to present the trees in language so free from technical terminology, and by illustrations so characteristic, that they may be recognized by "any person of ordinary information." This will certainly meet and stimulate the growing interest in trees, a purpose that is worth while. The authors are in an exceptionally favorable position to make such a book accurate rather than merely popular, and it is a good thing now and then for men who have the facts to give to the public something that can be relied upon. The identification of trees should now be as easy as the long popular identification of birds. The characters are drawn from foliage, flowers, and fruit, and they are presented in the free style of ordinary description, rather than in the compact style of taxonomy. The illustrations are from excellent sketches and photographs, and really illustrate. The economic value of the various trees is included, so that when the name of a tree is discovered, the inquirer is in a position to obtain much useful and interesting information concerning it. Of course any definition of a tree must be arbitrary, but the authors have liberally included all species known to become trees in habit (with "single erect stem or trunk"), even if they are almost always shrubs.—J. M. C.

MINOR NOTICES

Physiology of stomata.—LLOYD has given us a careful study of the behavior of the stomata in two desert plants, *Fouquieria splendens* and *Verbena ciliata*, made at the Desert Botanical Laboratory of the Carnegie Institution.[7] He addressed himself particularly to the question of the regulation of transpiration by stomatal movements, and furnishes conclusive evidence that the stomata in these plants, where there are no complications in the way of pits, plugs, or other contrivances, are not able to adjust the transpiration to the "needs" of the plants. Wide variation in the rate of transpiration is found, quite independent of the

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[5] BOT. GAZETTE **39**:301. 1905.

[6] BRITTON, NATHANIEL LORD, and SHAFER, JOHN ADOLPH, North American trees; being descriptions and illustrations of the trees growing independently of cultivation in North America, north of Mexico and the West Indies. Imp. 8vo. pp. x+894. *figs. 781*. New York: Henry Holt and Company. 1908. $7.00.

[7] LLOYD, F. E., The physiology of stomata. Imp. 8vo. pp. 142. *pls. 14. figs. 39*. Washington: The Carnegie Institution, Publication 82. 1908.

position of the guard cells, the maximum diffusion capacity of the pore being seldom (if ever) utilized. A rhythmic variation in the transpiration rate was found to be independent of the stomatal rhythm. As to the latter, LLOYD finds that, aside from the indirect effect of high relative humidity in reducing the water loss and so favoring the opening of the stomata, there is no relation between the humidity and the position of the guard cells. He finds no closure of the stomata in anticipation of wilting, but during wilting a slow closure, without the preliminary opening attributed to them by FRANCIS DARWIN.

LLOYD also attacked an interesting problem in the supposed photosynthetic activity of the guard cells. He finds evidence of amyloplastic but none of chloroplastic activity, and concludes that the movements of the guard cells are related to their accumulation and dissolution of starch derived from the chlorenchyma, rather than to any photosynthetic products of the guard cells themselves.

This is a careful and thorough piece of work, highly creditable to the laboratory from which it comes. The experimental evidence is now at hand supporting conclusions which have been held by some physiologists for some years as highly probable on purely physical grounds.—C. R. B.

The timbers of commerce.—A second edition of BOULGER'S *Wood*, revised and enlarged, has appeared.[8] It deals with 1000 kinds of wood, and includes most of those known in general commerce. The first part (pp. 121) discusses wood in general, under such topics as origin, structure, development, classification, defects, selection, uses, supplies, and tests. The second part presents the woods of commerce, giving in each case the source, character, and use. The 48 plates are from photomicrographs of sections, and are intended to show the distinctive microscopic features. Such a book is encyclopedic, and therefore for its purpose it is extremely useful. The demand for a second edition speaks well for the favorable reception of the first.—J. M. C.

Knuth's Handbook.—The second volume of DAVIS' English translation of this encyclopedic work has just been issued by the Clarendon Press.[9] The original volumes and the first volume of the translation were reviewed in this journal,[10] so that the general scope and character of the work have been noted. The present volume includes observations on flower pollination made in Europe and in the arctic regions, and is a great mass of observations upon species ranging through the natural orders, from "Ranunculaceae to Stylidieae." Such a book cannot be reviewed, for it is an encyclopedia. It can only be announced, and

[8] BOULGER, G. S., Wood, a manual of the natural history and industrial applications of the timbers of commerce. 8vo. pp. xi+348. *pls. 48*. London: Edward Arnold. 1908. $4.20.

[9] KNUTH, PAUL, Handbook of flower pollination. Translated by J. R. AINSWORTH DAVIS. Volume II. 8vo. pp. viii+703. *figs. 210*. Oxford: Clarendon Press. 1908. Half morocco 35*s*.; cloth 31*s*. 6*d*.

[10] BOT. GAZETTE **28**:280. 1899; **28**:432. 1899; **42**:494. 1906.

this translation should greatly stimulate observation in a field too much neglected by American botanists.—J. M. C.

Flora of Manchuria.—KOMAROV[11] has completed his *Flora of Manchuria* with the appearance of the second part of the third volume. The whole work contains 853 pages, and the last part includes the Sympetalae from Labiatae to Compositae. It is interesting to note that in presenting 18 families, 130 genera, and 336 species, only two new species are described—one a Scutellaria, the other a Saussurea. An appendix contains descriptions of two new species of Anemone. In this part much the largest families are Compositae (164 spp.), Labiatae (48 spp.), and Scrophulariaceae (43 spp.)—J. M. C.

Grasses of Louisiana.—R. S. COCKS,[12] Tulane University, has published a list of the grasses of Louisiana, based upon collections made during each season since 1897. The catalogue contains 290 species, which is said to represent 12 per cent. of the flora of the state. It is interesting to note that 11 species are known to find their northern limit in Louisiana; 9 species their southern limit; 10 species their western limit; and 10 species their eastern limit; while 5 species are given as occurring only in Louisiana so far as the United States is concerned.—J. M. C.

The western willows.—JONES[13] has published an account of the western Salicaceae, recognizing 53 species of Salix, with numerous varieties, and 8 species of Populus. The species have been studied in the field and the descriptions are compact and clear; so that the willows are presented as they actually appear in nature. A key to the species makes their recognition very direct, and the characters used are very obvious ones. Willows have been difficult to identify, and this presentation should be of much service.—J. M. C.

North American Flora.—The second part of Volume IX concludes Polyporaceae, by W. A. MURRILL, 32 genera being presented, 16 of which have recently been described by the author. In various genera 36 new species are described.—J. M. C.

NOTES FOR STUDENTS

Biometrical studies.—The close interrelation between fluctuations and the environment, especially those factors of the environment which in any way affect nutrition, has been recognized by many authors and overlooked or ignored by others. Several valuable contributions have been made to this subject. In a

[11] KOMAROV, V., Flora Manchuriae. Acta Hort. Petrop. **25**[2]:335–853. *pls. 4–16.* 1907.

[12] COCKS, R. S., Annotated catalogue of grasses growing without cultivation in Louisiana. Bull. 10. Gulf Biologic Station, Cameron, La.

[13] JONES, MARCUS E., The willow family of the Great Plateau. pp. 32. Salt Lake City, Utah.

paper read before the third Flemish Natural History Congress at Antwerp in 1899, MACLEOD[14] shows that in *Centaurea Cyanus* the mean numbers of rays and disk-florets are highest in heads which bloom earliest and that they fall continually as the flowering season progresses, the change in disk-florets being the greater. When individuals are considered, the terminal heads have the highest numbers, and each successive bud-generation has a less number than the preceding. Three series of cultures under different conditions of soil led to the conclusion that the first heads of each plant behave like the terminal heads of well-nourished plants; and that the last heads of each plant and of the season resemble the terminal heads of poorly nourished plants. There is no indication in this species that the Fibonacci numbers tend to predominate. This paper is not listed in DAVENPORT'S rather comprehensive bibliography, and was unfortunately unknown to me when I was investigating the seasonal variability in *Aster prenanthoides*. The conclusions reached for Centaurea are the same as those reached by me about a year later in Aster.

More recently MACLEOD and BURVENICH[15] have made an experimental study of the variability in the number of rays of *Chrysanthemum carinatum* and find essentially the same condition as in Centaurea, except that, unlike that species, *Chrysanthemum carinatum* shows the modes on the Fibonacci numbers, and as the mean number changes with the change of nutrition, the prominence of one mode is lessened as the neighboring one increases. The variation goes by steps or leaps from one favored value to the next. In discussing these "variation-steps" (*Varietietrappen*), three types of behavior are recognized: first, that in which the modes, whether one or several, agree with the terms of the relevant series, e. g., *Chrysanthemum carinatum*, 13 and 21; second, that in which the number of parts is constant or but very slightly variable and the values are those of the terms of the series, e. g., *Senecio Jacobaea*, 13, *S. nemorensis*, 5, *S. nemorensis octoglossus*, 8, etc.; third, the condition found in *Centaurea Cyanus* and in Aster in which the mode may fall upon any of the values lying between the terms of the series and in which as the mean values rise or fall the mode passes gradually through all the successive values. Seven variation-scales or series are recognized as fully demonstrated for one or more plant-characters; viz., (1) the Fibonacci series, 3, 5, 8, 13, 21, 34; (2) 5, 10, 15, 20 (carpels of Geranium); (3) 3, 5, 7 (leaflets of Trifolium); (4) 3, 6, 9 (flowers of Lonicera); (5) 2, 5, 8, 11 (flowers of *Cardamine pratense*); (6) 4, 8, 12, 16 (flowers of *Cornus mas*); (7) 4, 8, 16, 32, 64 (peristome of mosses). The author gives a lucid explanation of the cause for the existence of such series by referring them back to the period in

[14] MACLEOD, J., Over de veranderlijkeid van het aantal randbloemen en het aantal schijfbloemen bij de korenbloem (*Centaurea Cyanus*) en over correlatieverschijnselen. Botanisch Jaarboek **12**:40–74. 1900.

[15] MACLEOD, J., and BURVENICH, J. V., Over den invloed der levensvoorwaarden op het aantal randbloemen bij *Chrysanthemum carinatum* en over de trappen der veranderlijkheid. Botanisch Jaarboek **12**:77–170. 1907.

development when a single additional cell-division will determine the leap from one stage to the next. The causes which incite cell-division may proceed gradually and continually but not until they have increased to a certain required degree will the additional cell-division take place, so that the effect is more or less discontinuous, though the combination of causes may be continuous.

It is also shown by MACLEOD in the paper just mentioned and by DE BRUYKER,[16] both working with *Chrysanthemum carinatum*, that the usual rule, according to which the earliest heads have the highest numbers, may be reversed by increasing the nutrition after the earliest heads are blocked out, thus fully demonstrating that the normal change which takes place from the beginning to the end of the flowering season is dependent upon the regular decline in the nutritional conditions. This seasonal periodicity is strongly emphasized in another paper by DE BRUYKER.[17] He finds that the number of flowers in the umbels of *Primula elatior* follows the same law of seasonal change as that followed by Centaurea, Aster, and Chrysanthemum above mentioned, and now known to hold for a number of species. Specimens of Primula growing in dry places had a lower mean number of flowers in the umbels than plants growing in moister places. The variation-curves for this character differ under different conditions and at different times, but always display modes on the Fibonacci numbers, 3, 5, 8, and 13. Evidence is given to show that these multimodal curves are not due to the presence of heterogeneity in the race, but are referable to the fact that development proceeds by more or less discontinuous stages, whose value in any particular instance depends upon the external and internal environment.

The complete dependence of the values of fluctuating characters upon the environment has also been well shown by KLEBS[18] in a comprehensive investigation of variation in the floral organs of *Sedum spectabile*. To avoid the possibility of dealing with mixed races, KLEBS has confined his studies to groups of individuals formed by taking cuttings from a single original plant. Growing these in several different habitats, he finds that for the stamens there is a different type of variation-curve in each habitat, which remains fairly constant so long as the habitat remains unchanged. Stamens of Sedum show the first of MACLEOD'S three types of behavior, with 5 and 10 as the favored classes. The various habitats produce curves ranging from a monomodal curve with 5 as the mode and with very slight variability, through a bimodal curve with different relative values of modes 5 and 10, to monomodal curves with 10 as the mode. It is shown

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[16] DE BRUYKER, C., De gevoelige periode van den invloed der voeding op het aantal randbloemen van het eindhoofdje bij *Chrysanthemum carinatum*. Handel. 10de Vlaamsch Nat. u. Gen. Cong., Brugge, S. 1906. pp. 6.

[17] ———, De polymorphe variatiecurve van het aantal bloemen bij *Primula elatior* Jacq.; hare beteekenis en hare beinvloeding door uitwedige factoren. Handel. 10de Vlaamsch Nat. u. Gen. Cong., Brugge, S. 1906. pp. 29. *figs. 2.*

[18] KLEBS, G., Studien über Variation. Roux's Arch. **24**:29–113. *figs. 15.* Jy. 1907.

that not only variability itself, but the nature of the variation-curve and all of its "constants" are the product of the environment, and are a measure of the uniformity or lack of uniformity of environmental conditions. He concludes that there is no such thing as absolutely constant characters, and that the most constant may become quite variable under special conditions. There is no real distinction between continuous and discontinuous variability, if the question of heredity is left out of account, as both of these as well as transitions between them may be induced by changes of the environment. The applicability of QUETELET's law, which seems to indicate that variability is due to chance, depends upon the fact that the different values of the variable characters are determined by the coordinate action of several independently variable environmental factors. In an attempt to analyze the effective factors in the environment it is shown that the values of variable characters of Sedum increase directly as the quantity of carbohydrates increases and inversely as the quantities of available water and of salts increase. Chemical analysis of plants grown in the different habitats are presented in support of this view.

PEARL[19] has studied the variability of *Chilomonas paramecium* and *Paramecium caudatum* living under favorable and unfavorable conditions. He finds that the types of the two populations are significantly different, that of the less favored culture being smaller and relatively more slender. The variability was the same in the two cultures; but the curve of the well-fed culture was positively skew, while the poorly fed presented a nearly normal variation-curve. There is a correlation of 0.6 between length and thickness, and also a significant correlation between size and form, which is recognized as opposing DRIESCH's statement that proportionality is absolutely independent of size. In Paramecium, PEARL[20] also finds that there is a high degree of correlation or "assortative mating" between the two members of a pair of conjugating Paramecium, the coefficient ranging from 0.43 to 0.79 in respect to length, and 0.217 to 0.349 in respect to breadth. This strong tendency for like to mate with like is attributed to the inability of two individuals to unite successfully unless the mouths are of approximately the same size, the length of the mouth being closely correlated with other dimensional relations of the animal, particularly with the length. It is also found that the conjugants are differentiated as a class from the non-conjugating population, the former being smaller and less variable. From this the author concludes that conjugation tends to restrict rather than increase variability, and that the conjugant type bears much the same relation to the non-conjugants as that borne by the germ-cells of a metazoan to its soma. All evolutionary progress must rest upon changes in the conjugant type.

Of interest for the purely methodological side of biometry is a brief paper

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[19] PEARL, R., Variation in Chilomonas under favorable and unfavorable conditions. **5**:33–72. *figs.* 7. O. 1906.

[20] PEARL, R., A biometrical study of conjugation in Paramecium. Biometrika **5**:213–297. *figs.* 9. F. 1907.

by DE BRUYKER,[21] in which it is shown that the means and quartiles, and indeed all the ordinates, of the ogive, when calculated according to the methods of GALTON, are a half-unit too low, due to the fact that GALTON constructs his curve on the extremity of the classes instead of on their mid-values. The suggested correction brings a very close agreement between the median and the arithmetical mean. DARBISHIRE[22] gives an interesting popular discussion of correlation of the kind dealt with by biometers, using as his basis the dice-throwing experiment of WELDON, in which the relation between two successive throws of dice are the correlated quantities considered, when a certain number of dice from the first throw are left on the table to influence the result of the second. When none of the dice are left, the two throws are totally unrelated and the correlation is zero; and when all are left, so that the second entry is an exact repetition of the first, correlation is complete. Tables are given showing the actual results of throwing 12 dice in this way under every possible condition as to the number of dice left back to influence the second throw, thus beautifully illustrating the different degrees of correlation between zero and unity.—GEORGE H. SHULL.

Symbiosis.—A recent paper by KEEBLE of Reading and GAMBLE of Manchester, England, continues the investigations of these two naturalists into the subject of the symbiosis of plants and animals.[23] Of late years these two men have been almost the only ones continuing this study, which, as the literature shows, was being actively pursued fifteen or more years ago.

Convoluta roskoffensis is a simple flat-worm living between the tide-marks on the northern coasts of Europe. It is usually green, owing to the presence in its tissue of chlorophyll-containing cells, which have been diagnosed as one of the Chlamydomonadeae. They resemble the members of the genus Carteria, but for certain reason KEEBLE, the botanical collaborator, hesitates to place them positively in this genus. The life-history of both components of the association has been worked out. From this study it is clear that the flat-worm begins its existence free from green cells. There is no transmission of green cells or even of rudimentary chromatophores (plastids) from parent to egg. The green cells appear to be chemotactically attracted to the eggs and egg-cases. Pure cultures of the alga may often be obtained from egg-cases which have attracted and become partly filled with the green motile cells. By carefully washing in sea-water free from the alga, colorless Convolutas can also be obtained. These will remain colorless or will turn green according to subsequent treatment. If kept in sea-

[21] DE BRUYKER, C., Bemerkingen aangaande de Galton'sche curve. Handel. 10de Vlaamsch Nat. u. Gen. Cong., S. 1906. pp. 6. *figs. 2.*

[22] DARBISHIRE, A. D., Some tables for for illustrating statistical correlation. Mem. and Proc. Manchester Lit. and Phil. Soc. **51**:no. 16. pp. 21. diagrams 12. *1 dbl. pl.* 28 Je 1907.

[23] KEEBLE AND GAMBLE, The origin and nature of the green cells of *Convoluta roskoffensis*. Quart. Jour. Micros. Sci. **51**:167–219. *pls. 13, 14.* 1907.

water passed through a Pasteur-Chamberland filter, they will remain colorless; if kept in such filtered water to which a loopful of a pure culture of the alga has been added, they will turn green as completely as if they were kept in unfiltered sea-water. Apparently the young animals cannot be infected by cells removed from the bodies of older green Convolutas.

The green cells are reported to undergo degeneration in the tissues of the animal, especially as regards their nuclei, and to this is attributed the inability of the green cells to maintain an independent existence, or to thrive in cultures, or to infect young Convolutas, when removed from the bodies of older green ones. Whether under suitable conditions of culture, the algal cells may be able to complete themselves, to restore or to regenerate the deficient nucleus, to become rejuvenated, is not determined by these experiments.

The functions of the alga in the body of its host have been experimentally studied. The authors conclude that the alga, photosynthesizing sugar, is osmotically relieved of a considerable portion of this food. It must diffuse, if it remain unchanged, to adjacent cells containing less; but if the sugar be converted into starch, the animal cannot digest and absorb it so long as the algal cells themselves live. Soon after infection with the alga, the animal ceases to excrete its nitrogenous wastes, and these, absorbed by the alga, are converted by it into higher organic compounds, into nitrogenous foods. As many of these as are soluble also diffuse into adjacent animal cells. At this stage it would appear that both animal and plant profit by their association, the animal gaining a supply of sugar and of soluble nitrogenous food, the plant obtaining a steady supply of partly elaborated organic nitrogenous food-material. Later on, however, the animal ceases to ingest solid food, and there being no other source of nitrogenous food than the algal cells themselves, the animal proceeds to devour them in its own tissue. Thus it destroys its source of food and ultimately itself, but before this it breeds.

This association, therefore, is similar to that of fungus and alga in lichens, where the fungus component, incapable of photosynthesis, is forced to obtain non-nitrogenous food, saprophytically or parasitically. The excreta of the dependent component may manure the green plants, but if they do, the benefit is a dubious one, since it leads to destruction without issue.

A word may well be added to commend the ingenuity of the experiments, the care and thoroughness with which they seem to have been carried out, and the caution with which inferences have been drawn from them and stated.—G. J. PEIRCE.

Plant diseases.—A root disease of sugar cane, first described from Java in 1895 by WAKKER is the subject of a bulletin by FULTON.[24] It is characterized by canes of reduced size and weight, and by reduced leaf system. A large percentage of

[24] FULTON, H. R., The root disease of sugar cane. Bull. 100, La. Agric. Exp. Sta. Jan. 1908.

affected stalks die. These effects are traceable to deficiency in the root system. The lower leaf sheaths of affected canes do not fall away as they normally do, but remain cemented together by the whitish mycelium of a fungus, *Marasmius plicatus*, which occurs saprophytically upon decaying vegetable matter, and is also able to attack living tissues of low vigor and thus to become a parasite. The conditions favoring attack are summarized as follows: "Slowness of germination and early growth; improper cultural procedures; unsuitable soil; bad drainage; unfavorable seasonal conditions; the stubble crop." Preventive measures are: "careful cultivation; selection and disinfection of seed cane; resistant varieties; destruction of infected trash; resting land from cane."

A second bulletin by the same author treats diseases of pepper and beans.[25] He mentions a wilt of pepper caused by a non-sporing sclerotial fungus; the bean anthracnose due to *Colletotrichum lindemuthianum;* the bacterial blight due to *Pseudomonas Phaseoli;* and a disease caused by Rhizoctonia.

EUSTACE[26] has been experimenting on fruit rots. Tests were made of the ability of fungi to produce decay in cold storage. On March 9 several varieties of apples were inoculated with bitter rot (*Glomerella rufomaculans*), black rot (*Sphaeropsis malorum*), blue mold (*Penicillium glaucum*), brown rot (*Sclerotinia fructigena*), pink rot (*Cephalothecium roseum*), and Alternaria. The fruit was then placed in cold storage at a temperature of 31° F. When removed and examined on May 9, it was found that the only fungus which had caused decay was *Penicillium glaucum*. When removed to warmer temperature, all the other species developed and caused decay. Decay was not entirely prevented by a temperature from 35 to 56° F., and decay proceeded rapidly at a temperature of 48° to 69° F. *Sclerotinia fructigena* on the peach developed decay slightly in two weeks at a temperature of 32° F., when inoculated by puncture. Fruits inoculated by contact merely showed no decay at the end of two weeks under these conditions. Experiments were made to determine whether sulfur fumigation (1 oz. of sulfur to 25 cubic feet of space) would kill the spores of rot producing fungus. It was found that the spores of *Penicillium glaucum* can be killed this way, but that injury is caused to the apples themselves by the sulfur fumes. Careful notes were made concerning the development of apple scab, and it was ascertained that the scab of the apple can increase even when the scab in question is completely covered with a coating of the Bordeaux mixture.

Apple leaf spots have long perplexed the plant pathologist. One of them has at last been definitely proved to be caused by *Sphaeropsis malorum*.[27] The disease in question is characterized by circular or irregular reddish-brown spots

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[25] FULTON, H. R., Diseases of pepper and beans. Bull. 101, La. Agric. Exper. Sta. Jan. 1908.

[26] EUSTACE, H. J., Investigations on some fruit diseases. Bull. 297, N. Y. Agric. Exp. Station, Geneva, N. Y. Feb. 1908.

[27] SCOTT, W. M., and RORER, J. B., Apple leaf spot caused by *Sphaeropsis malorum*. U. S. Dept. Agric., Bur. Plant. Ind. Bull. 121, part 5. March 1908.

with slightly raised purplish margins, which attain a diameter of 3.5 to 13^{mm}. The mature spots are usually circular, but may become irregular. This disease has been variously attributed to Phyllosticta, Coniothyrium, and other causes. The present authors obtained pure cultures of *Sphaeropsis malorum* from all young spots which were studied. From older spots other fungi were obtained. Inoculations with this Sphaeropsis produced typical disease in 5–20 days upon leaves. The fungus causing the leaf spot in nature is probably derived from cankers on the branches which are frequently abundantly infested.—F. L. STEVENS.

Peat.—From the Geological Survey of Michigan there has recently appeared a volume on peat.[28] The work consists of three separate essays under the following titles: The ecology of peat formation in Michigan; The formation, character, and distribution of peat bogs in the northern peninsula of Michigan; Economics of peat. The author presents a classification of the Michigan peat deposits based upon (*a*) form of land surface, (*b*) method of development, and (*c*) surface vegetation, in which he seems to follow previous authors. The place taken by plants in peat formation depends directly upon their specific ecological demands, hence the floristic diversity which appears in the formation of peat in shallow depressions, upon flat areas and raised surfaces. Applying this principle, a thorough study is made of Mud Lake, which lies in the southern peninsula, and the successions are well traced out. The conclusion is reached that light is the principal factor controlling the development of peat through its limiting influence on the growth of plants, both below and above the water level. Physical and chemical characters of the substratum, temperature, aeration, mechanical and physiological effects of the wind, and competition are considered cooperating but secondary factors influencing peat development.

In the second essay the northern peninsula is made the basis of study, and the vegetation of a large number of lakes and ponds is described in some detail, and the conclusions reached from the study of the southern peninsula substantiated. A comparison of the conditions found in the two peninsulas leads to the statement that the noted variety of the sedge zone in the north is to be related to the fact "that in the cooler, more humid climate of the north, the shrubs mentioned are able to grow better in the water than they can in the south." From a study of "Algal Lake," a type of peat hitherto unrecognized, in this country at least, must be added to those which were known before, namely, algal peat, formed almost entirely of the remains of one-celled or few-celled plants.

To part I is appended a detail map of the original swamp area of the southern peninsula; while part II is likewise accompanied by a map of the original vegetation of the upper peninsula. These maps should prove of great service, both in this field and as a permanent record of a vegetation too fast suffering destruction.

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[28] DAVIS, C. A., Peat. From the report of the Geological Survey for 1906. pp. 95–395. Lansing. 1907.

It is to be regretted that fuller credit for most of the apparently new ideas is not assigned. One looks in vain for acknowledgment of the works of FRÜH and SCHRÖTER, GANONG, and TRANSEAU, whose studies have partially covered the significant results of DAVIS. The mention of these works in their proper places would relieve the book of much that might appear to be an original contribution. The greatest value of the book to the ecologist lies in its careful descriptions of various types of swamps and the detailed record of the distribution of peat-forming species. A complete index makes this material readily available. —LEROY H. HARVEY.

Endodermis of ferns.—The sporadic occurrence of the endodermis and the modifications it shows have been frequently remarked. A comprehensive study of this layer in the fern stem and leaf has been made by BÄSECKE,[29] whose contribution may be considered a companion paper to that of RUMPF[30] on the fern root.

Following this writer, BÄSECKE distinguishes (1) the primary endodermis, characterized by Caspary's band, and (2) the secondary endodermis, in which the cell walls are more or less thickened and suberized. The leaves of the eusporangiate ferns lack an endodermis, while those of Osmundaceae show only a primary layer; but most of the leptosporangiate ferns are well provided throughout the length of the leaf with a secondary layer. Anatomical and physiological studies show that food manufactured in a fertile leaf first supplies the sporangia, and any excess passes out through the vascular bundles. In rhizomes devoted to storage, only a primary endodermis is found, and in those which are active in propagating the plant a more or less impenetrable layer extends nearly to the growing point; hence the view is maintained that the secondary endodermis serves to prevent the escape of food from the vascular bundles while it is in process of transport.

The second part of the paper describes a reinvestigation of the question as to the occurrence of cork in the ferns, and the conclusion is reached that true cork is never present, but that substitutes are frequent, such as "metacutinized" walls of the outer cell layers. In this respect the ferns are less differentiated than the angiosperms. As to shedding of leaves, the author distinguishes three sorts of absciss layers, in contrast to earlier workers who were unable to find special structures connected with leaf fall. A classification of the various mechanical tissues of ferns concludes the paper.—M. A. CHRYSLER.

Protection from light.—BAUMERT reviews very fully[31] the many suggestions that appear in literature as to the function of various structures in protecting

[29] BÄSECKE, PAUL, Bieträge zur Kenntniss der physiologischen Scheiden der Achsen und Wedel der Filicinen, sowie über den Ersatz des Korkes bei dieser Pflanzengruppe. Bot. Zeit. **66**:25–87. *pls. 2–4.* 1908.

[30] RUMPF, G., Rhizodermis, Hypodermis, und Endodermis der Farnwurzel. Bibl. Botan. **62**:1904.

[31] BAUMERT, K., Experimentelle Untersuchungen über Lichtschutzeinrichtungen an grünen Blättern. Beitr. Biol. Pfl. **9**:83–162. *figs. 6.* 1907.

mesophyll cells from excessive light, and made exact measurements as to the efficiency of some of them. Light from a lamp, concentrated by a reflector, was allowed to fall upon the experimental leaves at an angle of 45° in a suitable moist chamber, and the differences measured by means of a thermocouple of needle form, inserted between two pieces of the leaf, and connected with a galvanometer. The cooling by evaporation as a source of error during the exposure (10–15 min.) could not be wholly avoided, but was assumed to be nearly uniform in the control and the experimental leaves.

The results show that hairy, scaly, shining, and glaucous leaves become less heated than the same leaves deprived of protection. A thick white coating of hairs, as in *Centaurea candidissima*, reduces the heating effect 37.5 per cent., shininess up to 30 per cent., and wax coating up to 13.6 per cent. A layer of water reduces it 19.2 per cent.; but this result seems more open to objection on the score of cooling by evaporation than the others, though the author takes it to be as valid as the rest. Reflection is held to be due in some Bromeliaceae to the inner epidermal wall, the cell acting like a concave lens, while epidermal cells that contain brown contents act as shades. The special value of the paper is in its application for the first time of quantitative methods, instead of deductive reasoning.—C. R. B.

Turgor and curvature.—The old problem has been again attacked by KERSTAN,[32] namely the question whether, under tropistic stimulation, there first occurs a variation in turgor that causes the curvature, both in growing parts and in motor organs. The evidence accumulating has been all against the idea, so far as concerns growth curvatures, and PFEFFER'S result with Hordeum has stood almost alone. KERSTAN adds his testimony that in most cases there is no acceleration of geotropic and heliotropic growth movements by a heightened turgor, and often the cells of the convex side become less turgid. When such curvatures are mechanically prevented, no one-sided or opposed turgor changes occur. Only in the nodes of some grasses does turgor rise on the convex side by 0.5–2 per cent. KNO_3. This is partly due to the interference with growth, partly to geotropic induction. But the growth reaction occurs in 2.5 hours, and the turgor reaction only after 15. Hence the turgor change does not cause the growth reaction. The geotropic movements by motor organs, as has already been sufficiently proved, are due wholly to turgor. The movements coincide in time with the turgor reaction, which appears to be produced by the migration of soluble materials from the concave to the convex side. The turgor differences do not appear on the clinostat, but turgor increases on all sides. In Marantaceae, however, a very slightly heightened turgor was found in curved petioles, and none could be observed in the imperfect organs of Malvaceae.—C. R. B.

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[32] KERSTAN, K., Ueber den Einfluss des geotropischen und heliotropischen Reizes auf den Turgordruck in den Geweben. Beitr. Biol. Pfl. **9**:163–213. 1907.

Mendel's law in violet hybrids.—BRAINERD[33] has given an interesting account of the offspring of supposed violet hybrids which show that certain characteristics behave according to MENDEL'S law. The characteristics observed were the color of the capsule, purple vs. green; and the color of seeds, brown vs. buff. The parents of his plants were supposed to be hybrids between *Viola hirsutula* and *V. papilionacea*. With respect to the two characters considered, the supposed hybrids should be di-hybrids. The number of F_2 offspring was only twenty-one, but all four of the possible combinations were present and the larger number showed the two dominant characteristics, purple capsules and brown seeds. Several F_3 families further demonstrated the Mendelian nature of these characteristics. The writer points out that Mendelian behavior in the offspring of the supposed hybrids demonstrates their hybrid character.—G. H. SHULL.

Age of Hepaticae.—CAMPBELL contends[34] that both the Hepaticae and Musci are old types, the perishability of their tissues and insufficient search accounting for the lack of known fossil remains. He finds in the wide distribution of certain genera of Marchantiales and Anacrogynae, and the cosmopolitan distribution of many of their species, evidence of the relative antiquity of the groups, since these plants are not adapted to rapid distribution. In proof of the latter he cites a few experiments, the character of the spores, and the fact that no Hepaticae have been found on Krakatau since the eruption, though Java with an abundant hepatic flora is in sight. Analogy from pteridophyte and spermatophyte distribution is adduced in support of this conception of the significance of bryophyte distribution. The weakness of the case is evident enough, but certainly it is as good as any, if one must speculate.—C. R. B.

Klinostats.—VAN HARREVELD finds[35] by chronographic tests that the klinostats in use at present are all defective in that they do not secure uniform rotation, especially when the axis of rotation is inclined or horizontal and the load is not exactly centered. The modern motor-driven forms are the least objectionable. This inequality of pace leads to definite and unexpectedly large geotropic responses and should therefore be eliminated. To obviate these difficulties, he has constructed an instrument upon new principles. It is driven by a weight automatically raised, and it is released under pendulum control by a rachet actuated by an electromagnet. The intermittent shock of stopping is reduced by vanes and a wheel train. Photographs and drawings are given and a full description is promised when the instrument has been exhaustively tested.—C. R. B.

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[33] BRAINERD, E., Mendel's law of dominance in the hybrids of Viola. Rhodora 9:211–216. *figs.* 2. 1907.

[34] CAMPBELL, D. H., On the distribution of the Hepaticae and its significance. New Phytol. 6:203–212. 1907.

[35] HARREVELD, PH. VAN, Die Unzulänglichkeit der heutigen Klinostaten für reizphysiologische Untersuchungen. Recueil Trav. Bot. Néerl. 3:173 ff. (pp. 144). *pls.* 3. 1907.

Cytology of the ascocarp.—According to FRASER,[36] *Humaria rutilans* (*Peziza rutilans*) shows some interesting features in the origin and development of the ascus. The ascocarp originates as a tangle of hyphae without any differentiated sex organs, but nuclei fuse in pairs and the cells containing the fusion nuclei form ascogenous hyphae. Nuclear divisions in the hyphae show sixteen chromosomes, as do also the first and second divisions in the ascus. These two divisions have the characters of the heterotype and homotype mitoses. The third division in the ascus has eight chromosomes. During the first mitosis in the ascus the two nuclei of the ascus fuse. The spores are delimited by radiations passing from the centrosome, but near the base of the spore vacuoles may take part in the process.—CHARLES J. CHAMBERLAIN.

Fossil cycadophytes.—WIELAND[37] has published a short preliminary account of his examination of some of the most famous specimens of the Mesozoic cycadophytes preserved in European collections. The specimens described are those of *Cycadeoidea etrusca*, *C. Reichenbachiana*, *Williamsonia gigas*, and *Anomozamites minor*. With his long training in the American forms, the author was able to detect features which had escaped previous notice, confirming his results as to the bisporangiate strobilus, the synangial microsporangia, the branching habit, etc. Of special interest are Williamsonia, which links up the Mesozoic with the modern cycadophytes, and Anomozamites, with its slender branching stem, small blade-like leaves, and small strobili, which is more suggestive of the dicotyledons than any known cycadophyte.—J. M. C.

Plant remains in Scottish peat bogs.—In continuing his studies of the Scottish peat bogs, LEWIS[38] has published the results of his investigations in the Scottish Highlands and in the Shetland Islands. Most interesting details are given in reference to the different zones, and the author summarizes the situation in the following statement: "All the Scottish peat mosses [bogs] show a definite succession of plant remains. The oldest, in the south of Scotland and the Shetland Islands, have an arctic plant bed at the base. This is succeeded by a forest of birch, hazel, and alder containing the temperate plants. A second arctic plant bed occurs above the 'lower forest,' and is overlaid in all districts (except the Hebrides and the Shetland Islands) by an 'upper forest' covered by several feet of peat bog plants."—J. M. C.

Blepharoplast and centrosome of Marchantia.—ESCOYEZ[39] has studied mitoses in the spermatogenous tissue of *Marchantia polymorpha* and *Fegatella*

[36] FRASER, H. C. I., Contributions to the cytology of *Humaria rutilans*. Annals of Botany **22**:35–55. *pls. 4, 5*. 1908.

[37] WIELAND, G. R., Historic fossil cycads. Am. Jour. Sci. IV. **25**:93–101. 1908.

[38] LEWIS, FRANCIS J., The plant remains in the Scottish peat mosses. Part III. The Scottish Highlands and the Shetland Islands. Trans. Roy. Soc. Edinburgh **46**:33–70. *pls. 4*. 1907.

[39] ESCOYEZ, EUD., Blépharoplaste et centrosome dans le *Marchantia polymorpha*. La Cellule **24**:247–256. *pl. 1*. 1907.

conica. The results are different from those of IKENO in regard to the blepharoplast and centrosome. He finds no centrosome in the mitoses preceding the mother cell of the spermatid, and believes that his failure to find one is due to the absence of such a structure and not to any defect in technique. Two blepharoplasts were observed first in the mother cell of the spermatid near the plasma membrane, far from the nucleus. ESCOYEZ does not consider the blepharoplast to be a true centrosome; yet he has not traced its origin, and whether it comes from within the nucleus or from a certain region of the cytoplasm is not settled. —S. YAMANOUCHI.

Sorus of Dipteris.—Miss ARMOUR[40] has studied material of Dipteris that included the younger stages of the sporangium. *D. bifurcata* showed simultaneous development of sporangia in the sorus, and *D. conjugata* sporangia of different ages. The former, therefore, conforms to BOWER'S "Simplices," and the latter to his "Mixtae." The three species seem to make a series in the form of the leaf, with *D. bifurcata* as the most primitive, and *D. quinquefurcata* in an intermediate position; parallel with this is the division of sori, leading to increase in their number and decrease in the number of sporangia in a sorus; and finally there is the progression from the "Simplices" type to the "Mixtae" type. This series is thought to have progressed from such a type as Matonia.—J. M. C.

Morphology of Symplocarpus.—ROSENDAHL has investigated the embryo sac and embryo of Symplocarpus, and an abstract of his results has been published.[41] The primordia of the flowers appear eighteen to twenty months before anthesis, the ovules being "formed" in the season (autumn) preceding pollination. The proembryo is of the massive type characteristic of the aroids, in this case becoming ovoid in form. There is a short suspensor, and the stem tip is organized in a groove of the proembryo which develops near the suspensor. The endosperm encroaches upon both integuments and even into the chalazal tissue; and in turn the massive proembryo ("protocorm") destroys the endosperm, so that finally the embryo is freely exposed in the cavity of the ovary.—J. M. C.

Proteases.—VINES, in his fifth paper on this subject,[42] reports that oily seeds, those of hemp in particular, contain proteolytic enzymes which act vigorously without restraint from the oil present. He succeeded in isolating, for the first time from plant tissues, "a protease that is essentially peptic in its properties, digesting fibrin to albumose or peptone, but not acting on albumose or peptone, whether produced by its own digestion of fibrin or added as Witte-peptone. The facts justify the conclusion that the hemp seed contains two proteases, the one a

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[40] ARMOUR, HELEN M., On the sorus of Dipteris. New Phytologist **6**:238–244. *figs. 11–14.* 1907.

[41] ROSENDAHL, C. O., Embryo sac development and embryology of *Symplocarpus foetidus*. Science, N. S. **27**:209. 1908.

[42] VINES, S. H., The proteases of plants. V. Annals of Botany **22**:103–113. 1908.

peptase, the other an ereptase. He hopes soon to arrive at a general conclusion as to the nature of "vegetable trypsin," which by his admirable researches so far seems resolvable into a peptase and an ereptase.—C. R. B.

Structure of chloroplasts.—This has long been in doubt, the current doctrine being that the ordinary chloroplast consists of a stongy stroma in whose meshes the chlorophyll is held as a green fluid. PRIESTLEY and IRVING show[43] that in the large chloroplasts of *Chlorophytum elatum*, *Selaginella Kraussiana*, and *S. Martensii* the chlorophyll is restricted to a peripheral zone, probably less than 1μ thick, where it is held in the meshes of a spongy stroma. This agrees with the arrangement theoretically best according to TIMIRIAZEFF. The authors also confirm the neglected observations of NÄGELI and TIMIRIAZEFF on the splitting of the chloroplasts in solutions of low osmotic pressure.—C. R. B.

Morphology of wheat.—ARTHUR H. DUDLEY,[44] in a presidential address before the Liverpool Microscopical Society, presented an account of floral development, sporogenesis, and embryogeny in wheat. A summary of his results is as follows: the archesporium of the microsporangium is a single row of cells, two or three divisions occurring before the mother-cell stage is reached; the archesporial cell of the megasporangium does not cut off a parietal cell, but produces directly the linear tetrad, the reduction number of chromosomes being eight; a large development of antipodal tissue occurs; and the embryo is said to be derived from the "apical cell only" of the proembryo.—J. M. C.

Scion and stock.—GUIGNARD has made another attempt to settle the question whether compounds peculiar to either scion or stock are able to migrate past the point of grafting.[45] When a plant which contains an HCN-glucoside is grafted on a plant which contains none, or conversely (GUINARD used *Phaseolus lunatus*, *Photinia serrulata*, and five species of Cotoneaster), there is no transfer of this glucoside in either direction. This adds one more bit to the negative evidence that is accumulating against the uncertain positive claims of such migration. The paper contains a good history of the question.—C. R. B.

Tolerance for salts.—Continuing their work on the relation between alkali soils and vegetation, KEARNEY and HARTER, testing pure solutions of various salts, find[46] that different species and even different varieties of the same species differ considerably in resistance to the action of magnesium and sodium salts.

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[43] PRIESTLEY, J. H., and IRVING, ANNIE A., The structure of the chloroplast considered in relation to its function. Annals of Botany **21**:407–413. *figs. 2.* 1907.

[44] DUDLEY, ARTHUR H., Floral development and embryogeny in wheat. Report Liverpool Micros. Soc. 1908 1–19. *pls. 1, 2.*

[45] GUIGNARD, L., Recherches physiologiques sur la greffe des plants à acide cyanhydrique. Ann. Sci. Nat. Bot. IX. **6**:261–305. *figs. 9.* 1907.

[46] KEARNEY, T. H., and HARTER, L. L., The comparative tolerance of various plants for the salts common in alkali soils. U. S. Dept. Agric., Bur. Pl. Ind., Bull. 113. pp. 22. 1907.

Seedlings grown from fresh seed are much more resistant than those from older seed. By different experimentation they confirm the findings of other observers as to the power of calcium salts (they speak of sulfate) to offset the toxic action of magnesium and sodium salts.—C. R. B.

Edwin James.—Students of taxonomy will be interested in a recent paper by PAMMEL,[47] which gives an account of Dr. JAMES, whose name is so intimately associated with the early explorations of the Rocky Mountain region. Not only numerous plants, but also a mountain peak bears his name, though the latter is now better known as Pike's Peak. Through papers found in the Parry herbarium, local biographical sketches, and information obtained directly from relatives, a very satisfactory account has been prepared, and the personality of JAMES is thus rescued for botanists.—J. M. C.

Conifers of China.—The late MAXWELL T. MASTERS left a paper on the distribution of conifers in China, which has just been published.[48] The total number of species known from China at the time of writing (June 20, 1907), inclusive of Formosa, was 87, distributed among 23 genera. In one table China and Japan are compared; the former containing 87 species, of which 42 are peculiar; the latter 48 species, of which 15 are peculiar. A large table shows the distribution of all the native species of China in the various regions of the empire as well as in neighboring countries.—J. M. C.

Nuclear division in Basidiobolus.—OLIVE[49] has studied nuclear and cell division both in the beaks and in the vegetative cells of Basidiobolus. The spindle is broad, cylindrical, barrel-shaped, and intranuclear. At the equatorial plate stage it shows three darkly staining regions, the chromatin plate at the center and two pole plates at the ends. The wall is formed as a ring, which begins at the periphery of the cell and closes in like an iris diaphragm, as in many algae, a mode of growth quite different from that described by FAIRCHILD in 1897.—CHARLES J. CHAMBERLAIN.

Variegation and infectious chlorosis.—Those who are interested in these problems will find useful an extensive paper by LINDEMUTH,[50] which embodies a precise and comprehensive exposition of the results of his studies on variegation, which go back as far as 1870, and have been continued with more or less vigor to

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[47] PAMMEL, L. H., Dr. Edwin James. Annals of Iowa **8**:161–185, 277–295. 1908. Also distributed as a separate.

[48] MASTERS, MAXWELL T., On the distribution of the species of conifers in the several districts of China, and on the occurrence of the same species in neighboring countries. Jour. Linn. Soc. Bot. **38**:198–205. 1908.

[49] OLIVE, E. W., Cell and nuclear division in Basidiobolus. Annales Mycol. **5**:404–418. *pl. 10.* 1907.

[50] LINDEMUTH, H., Studien über die sogennannte Panaschüre und über einige begleitende Erscheinungen. Landw. Jahrb. **36**:807–862. *pls. 8, 9. figs. 16.* 1907.

the present time. The paper includes a summary of many scattered contributions, and makes it clear that he had recognized, much earlier than BAUR, the infectious character of certain forms of variegation. He contends, rightfully, that chlorosis is an inept term.—C. R. B.

Sap rot of red gum.—VON SCHRENK[51] describes diseases due to fungi which infect the sapwood of red gum (Liquidambar) when the logs lie in the forest while wet, and continue to spread, to the destruction of the lumber when cut. *Polyporus adustus*, *Polystictus hirsutus*, and *Poria subacida* are the most frequent enemies, but there are a number of others. Sap rot may be prevented by hastening the drying or by coating the ends of the logs as soon as cut with hot coal tar. Similar diseases injure the heartwood of red gum and also affect the swamp oak and maple.—C. R. B.

Cytology of Microsphaera.—SANDS[52] has shown that during all stages in the life-history of Microsphaera a central body is differentiated as a permanent nuclear structure, which serves as a point of attachment for the chromatin. It is always extranuclear, never intranuclear, as claimed by MAIRE and GUILLIERMOND. The delimitation of spores is accomplished by the astral rays persisting from the third mitosis in the ascus. The work in this respect supports the conclusions of HARPER rather than those of FAULL.—CHARLES J. CHAMBERLAIN.

Morphology of Cornus florida.—MORSE[53] has investigated *Cornus florida* and found the following facts: the male gametophyte passes the winter in the two-celled stage; no walls separate the nuclei of the linear megaspore tetrad; the embryo sac probably passes the winter in the eight-nucleate stage, which persists until the last of May, when pollination occurs; the synergids are slender cones projecting far into the micropyle; the endosperm tissue is formed first in the micropylar end of the sac, and by the middle of July completely fills the sac.—J. M. C.

Fossil flora of Florissant.—COCKERELL[54] has enumerated the known flora of the Florissant shales (Miocene), including 106 genera, 45 of which occur in Colorado today. Of the genera not occurring now in Colorado, 36 occur in our eastern and southern states, the conclusion being reached that a flora similar to that of the Carolinian region occupied the Rocky Mountains during the Miocene. About 50 new species are described, including a Chara, a fungus, four ferns, and two gymnosperms.—J. M. C.

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[51] VON SCHRENK, H., Sap rot and other diseases of the red gum. U. S. Dept. Agric., Bur. Pl. Ind., Bull. 114. pp. 32. *pls. 8.* 1907.

[52] SANDS, M. C., Nuclear structure and spore formation in *Microsphaera alni.* Trans. Wis. Acad. Sci. **15**:733–752. *pl. 46.* 1907.

[53] MORSE, WILLIAM CLIFFORD, Contribution to the life history of *Cornus florida.* Ohio Naturalist **8**:197–204. *pl. 14.* 1907.

[54] COCKERELL, T. D. A., The fossil flora of Florissant, Colorado. Bull. Amer. Mus. Nat. Hist. **24**:71–110. *pls. 6–10.* 1908.

Trees and shrubs.—The second part of the second volume has followed[55] the first part promptly, and is devoted to species of Crataegus and Viburnum. SARGENT describes 10 new species of Crataegus, 7 of which are from Missouri; while REHDER presents and illustrates 13 species of Viburnum from China and Japan, 3 of which are new. The species of Viburnum are followed by a synopsis of the genus as displayed in eastern Asia, 65 species being recognized under 9 sections, and 9 of these species are new.—J. M. C.

Cytology of geotropism.—GEORGEVITCH contributes little that is new by his study of the cytology of roots of *Lupinus albus* when stimulated by gravity.[56] He correlates, much as NĚMEC did, though with differences in detail, the aggregation of the protoplasm and the position of the nucleus with geotropic stimulation, and so practically settles the fact that there are observable differences in the cell contents of normal and stimulated roots. It is not possible, however, to say what these mean.—C. R. B.

Sexuality in Ceratiomyxa.—OLIVE[57] has shown that the cleavage of the plasmodium of Ceratiomyxa to form spores is progressive and not simultaneous, as claimed by FAMINTZIN and WORONIN. Toward the close of the cleavage stage there is a fusion of nuclei in pairs, followed almost immediately by synapsis and two rapidly succeeding divisions, which are regarded by the author as reduction divisions, and give rise to four-nucleate spores.—CHARLES J. CHAMBERLAIN.

Dumortiera autoicous.—ERNST reports in a preliminary paper[58] that Javanese material of *Dumortiera trichocephala* (commonly) and *D. velutina* (sparingly) have both antheridia and archegonia on the same receptacle, though on different lobes. A single instance of the same thing has been observed (hitherto unpublished) by Dr. W. J. G. LAND, of the University of Chicago, in Mexican material of *D. hirsuta* collected at Xalapa, V. C.—C. R. B.

Anatomy of Isopyrum.—HOLM[59] has added *Isopyrum biternatum* to his anatomical records, including with the anatomical details a discussion of geographical distribution and generic limitations.—J. M. C.

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[55] SARGENT, C. S., Trees and shrubs. Illustrations of new or little known ligneous plants, prepared chiefly from material at the Arnold Arboretum of Harvard University. Vol. II. Part. II. pp. 57–116. *pls. 126–150.* Boston and New York: Houghton, Mifflin, & Co. 1908. $5.00.

[56] GEORGEVITCH, PETER M., Cytologische Studien an den geotropisch gereizten Wurzeln von *Lupinus albus.* Beihefte Bot. Cent. **22**[1]:1–20. *pl. 1.* 1907.

[57] OLIVE, EDGAR W., Cytological studies on Ceratiomyxa. Trans. Wis. Acad. Sci. **15**:753–773. *pl. 47.* 1907.

[58] ERNST, A., Ueber androgyne Inflorescenzen bei Dumortiera. Ber. Deutsch. Bot. Gesells. **25**:455–464. *pl. 13.* 1907.

[59] HOLM, THEO., *Isopyrum biternatum* T. & G.; an anatomical study. Am. Jour. Sci. IV. **25**:133–140. *figs. 3.* 1908.